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CEREBROSPINAL FLUID, ITS PHYSIOLOGY AND DIAGNOSTIC EVALUATION

C. DE CHENAR*

The purpose of this study is to outline practical knowledge concerning the cerebrospinal fluid, supplemented by the experience obtained through the application of the author's $CuSO_4$ test. For the last five years, this test has been used in clinical laboratories in America, Europe and Asia.

Within the continuous cavity of the skull and the spinal canal, the brain and the spinal cord are suspended floating in the cerebrospinal fluid. Thus, the central nervous system, which is highly sensitive to vibrations and temperature changes, is provided with the best possible protection against mechanical and thermal injuries.

The existence of the human cerebrospinal fluid was disclosed by Cotugno and Magendie, early in the XIX. century. The understanding of its origin, composition and physiological significance, however, was delayed, for more than a hundred years. In 1905, the discovery of the Treponema pallidum in the brain parenchyma in cases of general paresis (dementia paralytica progressiva) by Schaudinn, was the decisive impulse toward the diagnostic evaluation of chemical, physico-chemical and microscopical findings in the cerebrospinal fluid. Since the contribution of Lange in 1912, on the basis of his colloidal gold precipitation of the liquor proteins, the colloid-chemical properties have become known to a considerable extent. The progress of organic chemistry was prerequisite for further information on the cerebrospinal fluid.

Origin and Function

In the production of the cerebrospinal fluid, evidently, the surrounding tissues participate. Observations on pathological occlusions, partial or total, and experiments with dyes and colloidal materials, point toward the plexus chorioideus, ependyma, tela arachnoidea and periradical lymphatic spaces, as the structures where various components take their origin.

Secretion by the plexus chorioideus was demonstrated by Dandy. He observed that in case of unilateral occlusion of the foramen interventriculare of Monro, ipsilateral, univentricular, hydrocephalus developed. In this process, the biological permeability of the contributing tissue elements is the basic factor, by dialysis and/or active secretion of the components. Besides the productive function, the plexus chorioideus has a limited resorptive activity.

The cerebrospinal fluid is in constant circulation (1) from the ventricular space toward the Pacchionian granulations where it is absorbed into the venous blood of the sinuses and (2) from the periradicular lymphatic channels toward the subarachnoidal space. The studies of Haymaker and Kernohan have established our knowledge of this factor. They explained the increased amount of cerebrospinal fluid protein in Guillain-Barré's syndrome by the penetration of inflammatory products along the roots of the spinal nerves. The subperineural endothelial spaces, marginal sinus of Renaut, have a one way communication with the subarachnoidal space.

The leptomeninges and the ependymal epithelium also participate in the production of cerebrospinal fluid, but, evidently, under experimental or pathological conditions only.

The internal pressure within the liquor containing spaces varies on a small scale. The nervi proprii of the meninges are mainly responsible for maintaining this pressure. Upon intravenous administration of hypertonic solutions, the pressure temporarily decreases. Besides physical and colloid chemical factors, a hormonal regulation is the physiological way to control the intrathecal pressure: hypersecretion of the neurohypophysis increases, while that of the thyroid gland decreases it by influencing the production.

Nutritive, noxious or immune biological substances reach the parenchyma of the central nervous system through the selectively permeable structures of the mesenchymal filter built up of endothelial and adventitial cells, beyond which the 'membrana limitans gliae perivascularis' takes over the function of forwarding those substances, via astrocytes or oligodendrocytes to the neuronal elements. This system is the blood-brain barrier.

A smaller amount of substances passes through the other selectively permeable layer of the ectodermal filter, represented

by the chorioidal epithelium, as the blood-liquor barrier. The physiological significance of these filters is, briefly, the selection and the control of accumulation of vital chemical compounds for the central nervous system, in some instances even in a considerably higher concentration than in the blood plasma. On the other hand, these filters block the way of harmful materials as long as the balance stays on the side of the defense. A nutritive function, if any, of the cerebrospinal fluid cannot be decisive as its removal and substitution with air does not influence the functional capabilities of the brain, while the suppression of the blood supply does.

The removal of metabolites of the neural parenchyma and of eventual neurophagia centers around the unique lymphatic system of this organ, in the perivascular and superficial channels. The function of the perivascular spaces of Virchow-Robin is still unknown, whether they conduct materials from the subarachnoidal spaces to the brain or in the opposite direction, if they do so at all. A brain-liquor barrier might regulate the exchange at this level.

The chemical composition of the cerebrospinal fluid, with regard to the percentual contents of dialysable materials, differs from that of the blood serum so that the equilibrium of Donnan, concerning osmotic and hydrostatic pressure, does not apply in every way. Instead of a semipermeability, a 'physiological permeability' occurs according to Walter. This means an active, both qualitative and quantitative, selection and concentration of the actually needed substances, following a purposeful pattern. The concentration of some substances is different at various points in the cerebrospinal fluid, from the intraventricular to the lumbosacral level, as shown in Tables 2 and 3. Sachs et al. conclude that "substances in the cerebrospinal fluid spread by diffusion, but this diffusion is influenced to a great extent by gravity." Experiments of Bering with D_2O, as well as those of Walace and Brodie with bromide and iodide clarify further details of the distribution of materials in the cerebrospinal fluid. They all emphasize the minor importance of the ventricular source of production. Gilligan et al. state, in their final conclusion concerning the mechanism of production and distribution of substances that "the cerebrospinal fluid is not a dialysate or a simple ultrafiltrate." Among hormones occurring in the cere-

brospinal fluid, that of the neurohypophysis is most significant. Its concentration fluctuates, or it even disappears, under certain conditions. Following menstruation, it is at its highest value. Toward the end of pregnancy, it decreases, after delivery, it gradually increases. In cases of idiopathic and also symptomatic epilepsy, it is entirely absent in more than half of the patients. Its presence inhibits the bioelectric discharge of seizures. Hormones of the adenohypophysis may be demonstrated in pregnancy, in cases of mola hydatidosa and chorionepithelioma.

Constituents

The biological, experimental and diagnostic analysis of the cerebrospinal fluid requires the determination of certain physical and chemical data. The most frequently needed constants are summed up in Tables 1 and 2. From the clinical viewpoint, it is well to know that the concentration of proteins is at its highest level during the first year of life, and undergoes a gradual decrease thereafter. Just the opposite is the case with the corpuscular elements toward senium.

Several permanent constituents, as proteins and lipoid phosphorus, show different concentrations at various levels. A similar level difference may often be found in the concentration of

TABLE 1

Physical Constants

Color	water clear
Quantity	100 – 200 ml
Amount produced daily	700 ml
Specific gravity	1004 – 1008
Viscosity	1.01 – 1.06
pH	7.4 – 7.5
Lowering of freezing point	—0.55°C – —0.57°C
CO_2 combining power	40 – 60 vol. %
Refractometer index	1.335
Interferometer index	1370
Cell number, infant	0 – 20 per mcl
Cell number, adult	0 – 9 per mcl
Pressure, newborn	30 – 80 mm H_2O
Pressure, child	50 – 100 mm H_2O
Pressure, adult	70 – 200 mm H_2O

reagins of complement fixation reactions, where the intraventricular fluid might be negative while the cisternal and lumbar specimens yield a more and more positive reaction for syphilis. Just the opposite is the case with dextrose: its concentration gradually decreases downward in the liquor-containing space. This is to be kept in evidence in cases of meningitides where hypoglycorrhachia can be pathognomonic.

All these chemical compounds have their definite and specific task in the maintenance of the physiological equilibrium of the cerebrospinal fluid and they show reactive changes in case of disturbances caused by endogenic or exogenic noxious factors. Pathological reactions may arise in the cerebrospinal fluid that either evoke disarrangements in the proportion of some chemical constituents and in their interrelation, or provoke compounds previously not present. Certain alterations in the central nervous system, of its parenchyma, interstitium or meningeal elements result in a consecutive increase, decrease or qualitative modification of different groups of the constant components of the cerebrospinal fluid. In cases of degenerative, involutive, nutritive or circulatory changes and in diseases caused by microorganisms, one or more of the chemical constituents suffer a reactive alteration which might be characteristic and even pathognomonic of the pathogenic agent.

Proteins, Colloidchemical Details

From the viewpoint of chemical research and diagnostical examination of the cerebrospinal fluid, the proteins assume the foremost biological and pathological importance. They may be altered and give the first useful sign in the great majority of cases, although they are traceable only in small amounts under physiological condition. The proteins and the cerebrospinal fluid itself, as their carrier, have the physicochemical properties of colloids. The proteins behave as the dispersed phase within the basic liquid of the cerebrospinal fluid which is their dispersion medium. In the size of the colloidal particles, there are gradual differences between wide limits below and over 100 millimicrons. The colligative properties of the cerebrospinal fluid, as their freezing temperature, the osmotic pressure and the viscosity, depend upon the physical quality of the dispersed phase and upon

TABLE 2

Chemical Constants

Total protein	18–38 mg/100 ml
Albumin	15–30 mg/100 ml
Globulin	3.5– 9 mg/100 ml
Euglobulin	traces, if any
Fibrinogen (under path. cond. only)	none
Albumin/globulin ratio	2–5
Protein quotient	0.2–0.45
Total N	15–22 mg/100 ml
NPN	12–20 mg/100 ml
Amino acids	below 1 mg/100 ml
Urea	6–15 mg/100 ml
Uric acid	0.3–1.3 mg/100 ml
Indican (under path. cond. only)	none
Creatinin	1–1.5 mg/100 ml
Xanthoprotein	5–9 mg/100 ml
Dextrose, ventricular level	55–90 mg/100 ml
Dextrose, cisternal level	50–70 mg/100 ml
Dextrose, lumbosacral level	45–65 mg/100 ml
Lactic acid	8–15 mg/100 ml
Cholesterol	0.07–0.2 mg/100 ml
Lecithin	22 mg/100 ml
P	1.5–2.7 mg/100 ml
P, inorganic	1.4–2.6 mg/100 ml
P, lipoid, ventricular level	0.005 mg/100 ml
P, lipoid, cisternal level	0.01 mg/100 ml
P, lipoid, lumbosacral level	0.02 mg/100 ml
Chlorid, as NaCl	700–750 mg/100 ml
Nitrates	traces
Na	260–330 mg/100 ml
Ca	4.4–5.8 mg/100 ml
NH_4	below 0.1 mg/100 ml
S	1 mg/100 ml
Mg	1–1.5 mg/100 ml
Fe	traces
Vitamin C	0.2–1.7 mg/100 ml
Neurohypophysis hormone, ventr. level	0.0009 mg/100 ml
Neurohypophysis hormone, lumbar level	0.0006 mg/100 ml

the chemical composition of the whole system. The freezing point is about $-0.56°$ C, corresponding to that of a $\frac{1}{3}$ molar solution with an osmotic pressure of approx. 8 atm. The osmotic pressure of the cerebrospinal fluid is practically determined by the NaCl proportion and, only an irrelevant part of it is given

by other electrolytes. The osmotic effect of the proteins may be disregarded at this point, as their removal does not result in a considerable change of the balance. The viscosity, in turn, depends essentially on the concentration of the proteins. The correlation is similar to that of the variables of a quadratic (parabolic) function, if the dispersity and the proportion of the protein components are constant. Kjeldahl's method gives an average of 1.04 for the cerebrospinal fluid, while that for the blood is about 1.80.

The cerebrospinal fluid, being a colloidal system, has an appropriate static electric charge, depending upon two factors: (1) the chemical constitution of the colloid and (2) the presence of the electrolytes. The homonymic electric charges, also here, repulse and the heteronymic ones attract one another. It is possible to change the electric charge of the colloidal particles and, in consequence, to influence the fundamental properties of the cerebrospinal fluid as a colloid. The greatest possible change is the neutralization of the dispersed phase. This occurs in case of electronegative particles by the addition of cations, and in case of electropositive particles, by that of anions. An adequate proportion of the ions will decrease the original electric charge to the limit of the isoelectric point where flocculation of the dispersed phase will take place. While approaching the isoelectric point, the originally balanced concentration of the homonymic, each other repulsing, charge of the colloidal particles is more and more hindered and finally paralyzed. The static electric charge of colloids which contain proteins is like that of amphoteric solutions. In the presence of acid electrolytes, the protein molecules join the H-ions, while in case of basic electrolytes, they attach to the OH-ions, for possible neutralization.

In other aspects, the cerebrospinal fluid is like a hydrated emulsoid and it is dehydratable by means of adequately concentrated solutions of salts when the dispersed particles are deprived of their bound water and the previously separated particles adhere and gradually flocculate. The hydration of colloids is the resultant of several components, as (1) the chemical constitution of the colloid, (2) the dimension of the dispersed particles, (3) the static electric charge and (4) the presence of additional electrolytes. The grade of hydration is a basic factor for the colloidal state. The less hydrated, *i.e.*, the more hydrophobic the protein

particles are, the more instable is the colloidal system and flocculation results more easily. The globulin of the cerebrospinal fluid is extremely hydrophobic.

The physiologically important proteins, present in the blood and in the cerebrospinal fluid, include a large variety of different types and groups. The proteins themselves are built up of rather large molecules. Their molecular weight varies from 30,000 to 150,000 and their average diameter is about 4 millimicrons. The cerebrospinal fluid contains about 0.30% of the plasma protein which is about 25 mg per 100 ml out of the 7,000 mg per 100 ml. The osmotic equilibrium between the blood plasma and the cerebrospinal fluid is maintained by the high Cl percentage of the latter, about 720 mg per 100 ml. The non-diffusible colloidal particles of the cerebrospinal fluid, being in lower concentration than those of the blood serum, retain on their side the ions in a higher concentration, in order to obtain an equilibrium.

The liquor-proteins have their source in the same structures from where the cerebrospinal fluid originates: (1) the cerebral and spinal arachnoidea, (2) the chorioid plexus, (3) the ventricular ependyma and (4) the neuroparenchyma, incl. the cerebrospinal nerves. These proteins, like those of the blood, have diverse physiological functions and they are the specific carriers of chemical compounds, infiltrating into the cerebrospinal fluid. These proteins act by their characteristic affinity to molecules of exogenous origin, or to molecules deriving of complex compounds present in the cells and in tissues under physiological conditions. These colloidal proteins have the ability to establish a loose connection with nonphysiological chemical compounds also. They do not separate before reaching the organs secreting or excreting them out of the organism, together with metabolites. The pathological chemical compounds, even in the cerebrospinal fluid, arise from interference between noxious agents and defensive reactions.

In analyzing the colloidal proteins which participate in the defensive reactions of the cerebrospinal fluid, one finds that these do not represent a uniform order of proteins, but they appear in groups of molecules of various dimensions and structures. Therefore, their classification is rather difficult. This, on the basis of their amino acids, as to the number and structural order within the protein molecule, is of little practical value. More useful is

their physico-chemical classification, mainly as of components of a colloidal system.

The characteristic qualities of the liquor-proteins might be examined if the various groups are separated. The first reagent to be applied is usually ammonium sulfate. In accordance with the increasing concentration of this salt in distilled water, added to the cerebrospinal fluid, at first the larger molecules precipitate, being the least stable colloidal particles. The cerebrospinal fluid does not contain fibrinogen, therefore, the first fraction among its proteins is the globulin group. It precipitates when the concentration of the reagent is raised to 28—32%. The first member of this group is euglobulin, present in very small amount, if any. It is insoluble in distilled water.

The next fraction, pseudoglobulin, precipitates at a concentration about 32—50% of the ammonium sulfate and is regarded to be a water soluble protein. Its molecules are much smaller than those of any of the previous fractions. For their precipitation, it is not sufficient to eliminate the electrolytes, but they must be deprived of their bound water, attached to them by the oncotic or colloid-osmotic pressure. This pressure is about 25 Hg mm for a 1% solution in water. The pseudoglobulins are composed mainly of glycine, tryptophane and choline and contain a minor proportion of lysine and cystine. As far as they are present, as gamma globulin, their physiological task in the cerebrospinal fluid consists of the binding and transportation of immune bodies and, in a specific group of diseases, in the conjunction of complement. Their concentration increases in case of irritation, in several intoxications, inflammations, neoplasms, bacterial and parasitic infections and in a large scale of endogenous and exogenous alterations. The concentration of globulins varies from about 10 to 30% of the total protein contents. The actual concentration in the human cerebrospinal fluid depends also upon the anatomical region of the central nervous system. Table 3 gives the details.

The last fraction to precipitate at the complete saturation of the cerebrospinal fluid with ammonium sulfate, is the albumin group. These proteins have but a minor physiological significance. Their concentration amounts to about 15—25 mg per 100 ml. One usually determines their proportional correlation to the

TABLE 3

Level Differences in Protein and Cell Contents

Level of origin	Total protein mg per 100 ml	Globulin mg per 100 ml	Albumin mg per 100 ml	Cells p. microl.
Ventricular	10–16	1–4	8–14	0–1
Cisternal	16–20	1–6	14–16	0–2
Lumbar	16–28	2–8	14–25	0–9

globulins and gives the result as the protein quotient, or its reciprocal value, the albumin/globulin ratio.

Laboratory Findings, Diagnosis

Obviously, the globulin fraction has the most evident significance in the defensive mechanism of the cerebrospinal fluid. Its reactive changes are the most useful factors in tracing the interrelation between noxious agents and the organism, particularly the central nervous system. Therefore, the routine examination of the cerebrospinal fluid, by means of the various protein reactions, aims to obtain information as to the changes of the globulins.

For practical, clinical, purposes, the first and often only step taken to determine an evtl. globulin elevation is a qualitative or semi-quantitative test. For several decades, the tests of Pándy, Nonne-Apelt-Schumm and Weichbrodt have been used, with proper evaluation. The test of Donovan-Foley-Moloney is a later addition, but already time-tested and dependable.

Finally, the introduction of $CuSO_4$ as reagent in the laboratory analysis of cerebrospinal fluid, by the author of this article, had supplemented since 1954 the series of globulin reactions with a simple semi-quantitative test. It is sufficiently sensitive for tracing the changes caused by meningismus, viral, bacterial or specific meningoencephalitis, degenerative, demyelinating and involutional diseases, as well as by metabolistic or exogenous toxic conditions. It proved to be most sensitive for syphilitic alterations, particularly in cases of long duration where, after periodic treatments, most other reactions fail to show evidence of the inactive stage.

Table 4 summarizes, in a practical way, the changes of the

cerebrospinal fluid in the more frequently encountered pathological conditions.

Summary

A review of the physiology and pathology of the cerebrospinal fluid correlates theoretical explanations with actual findings. A discussion of the origin of the cerebrospinal fluid is followed by an analysis of pathological conditions as they arise in various diseases. Routinely, as well as occasionally, encountered laboratory findings are summarized in tables. The information has been collected from the literature and from the author's experience of over two decades. There is reference to the $CuSO_4$ test, the latest semi-quantitative reaction in the diagnosis of the cerebrospiral fluid.

BIBLIOGRAPHY

Bering, E. A., Jr.: Water Exchange of the Central Nervous System and Cerebrospinal Fluid. J. Neurosurg. Vol. 9, 3: 275, 1952.

Cohn, D. J., I. Kaplan, and A. Levinson: A Study of Calcium and Phosphorus in Cerebrospinal Fluid. J. Lab. & Clin. Med. Vol. 24, 6: 609. 1939.

Crosby, R. M. N. and G. L. Weiland: Xanthochromia of the Cerebrospinal Fluid. A.M.A. Arch. Neurol. & Psychiat. 69: 732, 1953.

Dandy, W. E.: Hirnchirurgie, J. A. Barth, Leipzig 1938.

de Chenar, C.: Copper Sulfate as Reagent in Cerebrospinal Fluid Analysis. Texas Rep. Biol. & Med., 12: 453–463, 1954.

Eskuchen, N.: Liquoruntersuchung. Neue Dtsche Klin., Bd. 6. Urban & Schwarzenberg, Berlin & Wien, 1930.

Exton, Wm. G. and A. R. Rose: Clinical Determination of the Albumin Globulin Ratio in Spinal Fluid. J.A.M.A. 96: 36, 1931.

Fremont-Smith, F. and M. E. Dailey: The Human Cerebrospinal Fluid. Paul B. Hoeber, Inc., New York, 1924.

Fremont-Smith, F., M. E. Dailey, H. H. Merritt, and M. P. Carroll: The Equilibrium between Cerebrospinal Fluid and Blood Blasma. Arch Neurol. & Psychiat., 25: 1271, 1931.

Gilligan, D. R., M. C. Volk, and H. L. Blumgart: Observations on the Chemical and Physical Relation between Blood Serum and Body Fluids. New England J. Med. Vol. 210, 17: 896, 1934.

Haymaker, W. and J. W. Kernohan: Landry-Guillain-Barré Syndrome. Medicine, Detroit, 28, 59–141. 1949.

Jessen, N.: Cytologie du liquide céphalo-rachidien normal chez l'homme. Masson & Cie., Paris, 1936.

Kaplan, I., D. J. Cohn, A. Levinson, and B. Stern: The Sources of the Enzymes of Normal and Pathologic Cerebrospinal Fluid. J. Labor. & Clin. Med. Vol. 25, 5: 495, 1940.

Katzenelbogen, S.: The Cerebrospinal Fluid and its Relation to the Blood. The Johns Hopkins Press, Baltimore, 1935.

Levinson, A.: Cerebrospinal Fluid in Infants and Children. Med. Clin. North America. Vol. 30, 1946.

TABLE 4

	Total Protein	Albumin	Globulin	Protein Quot.	N.P.N.	Urea	Uric Acid	Aceton	Indican	Creatinine	Xanthoprotein	Dextrose	Lactic Acid	Cholesterol	Lecithin	NaCl (Cl)	Bilirubin	Delta (°C)	Coagulation	Hemolysin React.	Lues React. Blood	Lues React. CspFl.	Coll. Gold Low Dil.	Coll. Gold High Dil.	Cells per microl.	Microorganisms	Pressure	Remarks
Physiological aver.	20	14	6	0.5	20	25	1.5	....	....	1	....	60	....	0.2	22	725	....	....	....	....	....	....	....	....	3	....	200	....
Meningismus	30	19	11	0.6	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	8	....	210	....
Encephalography	32	25	7	0.3	....	....	....	....	....	....	....	85	....	....	....	....	....	....	....	....	....	....	±	....	520	....	250	....
Contusion	30	20	10	0.5	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	+	....	10	....	255	....
Nonne-Froin syndr.	75	25	50	1.6	....	....	....	....	....	....	+	....	....	0.3	25	....	....	....	+	....	....	....	....	±	5	....	....	....
Hypertension	45	32	13	0.4	....	....	....	....	....	....	....	85	....	....	....	....	....	....	....	....	....	....	....	....	....	....	280	....
Arteriosclerosis	40	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	5	....	....	....
Apoplexy,ventric.	3000	....	....	....	....	....	....	....	....	....	+	90	....	....	....	....	....	....	+	....	....	....	....	+	....	....	350	(1)
Encephalomalacia	50	....	....	....	....	....	....	....	....	....	....	110	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Sinus thrombosis	400	....	....	....	....	....	....	....	....	....	+	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Meningitis, septic	700	350	50	1.0	....	....	....	....	....	2.4	+	30	40	10	....	650	....	....	....	....	....	....	....	+	2000	+	500	....
Mening., csp. epid.	200	120	80	0.7	....	....	....	....	....	....	....	40	....	....	....	....	....	....	....	....	....	....	....	+	1500	+	400	....
Mening., tubercul.	500	280	220	0.8	....	....	....	....	....	....	....	30	....	....	....	580	....	....	+	+	....	....	+	....	100	+	250	....
Cerebral abscess	250	123	127	1.1	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	+	4000	+	400	....	
Poliomyelitis	300	....	....	....	....	....	....	....	....	....	....	80	....	....	....	....	....	....	....	....	....	....	±	±	....	....	....	(2)
Encephalitis leth.	100	55	45	0.8	....	....	....	....	....	....	....	95	....	....	....	....	....	....	....	....	....	....	±	....	140	....	....	....
Postencephalitis	50	....	....	....	....	50	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	7	....	....	....

Herpes zoster	40	25	15	0.6	....	....	....	....	....	....	....	55	....	....	....	....	....	....	....	....	....	....	±	....	75	....	....	....
Syphilis, early	39	25	14	0.5	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	+	±	±	....	10	....	230	....
Gen. Paresis	90	40	50	1.2	....	40	....	....	....	....	....	....	....	40	....	....	....	....	....	+	+	+	+	....	150	....	230	....
Taboparalysis	60	35	25	0.8	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	±	+	+	....	180	....	220	....
Tabes	65	40	25	0.7	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	±	±	+	+	90	....	180	....
Lues csp. mening.	200	....	....	....	....	....	....	....	....	....	+	40	....	....	....	....	....	....	....	....	....	+	+	....	1400	....	350	....
Lues csp. vascul.	70	40	30	0.8	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	±	+	+	+	80	....	220	....
Lues csp. gumm.	40	20	20	1.0	....	....	....	....	....	....	....	....	....	50	....	....	....	....	....	....	±	±	+	....	50	....	....	....
Mult. sclerosis	30	20	10	0.5	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	±	±	30	....	210	....
Tumor, intracran.	100	....	....	....	....	....	....	....	....	....	±	90	....	....	....	....	....	....	....	....	....	....	±	....	100	....	400	....
Diabetes mellitus	20	....	....	....	....	....	....	±	....	....	....	110	....	....	....	....	....	–0.7	....	....	....	....	±	....	7	....	....	....
Icterus, obstructive	20	....	....	....	25	....	....	....	....	....	....	....	....	....	....	....	+	....	....	....	....	....	....	....	....	....	....	(3)
Funicular myelosis	30	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	8	....	....	....
Nephritis	45	....	....	....	230	400	....	....	....	....	....	....	....	....	....	800	....	....	....	....	....	....	....	....	30	....	....	....
Nephrosclerosis	30	....	....	....	....	....	....	....	....	....	±	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Uremia	50	....	....	....	150	....	3.5	+	+	3	+	95	....	....	....	820	....	–0.7	....	....	....	....	....	....	200	....	480	....
Cardiac incompens.	20	....	....	....	....	....	....	....	....	....	....	....	....	....	....	770	....	....	....	....	....	....	....	....	....	....	....	....
Epilepsy	30	20	10	0.5	....	....	....	....	....	....	....	80	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	(4)
Saturnismus. Plumbism	80	....	....	....	....	....	....	....	....	....	....	75	....	....	....	....	....	....	....	....	....	....	±	....	15	....	255	(5)

Remarks: (1) Hemorrhage. (2) Number of cells may increase in meningeal involvement up to 1000. (3) Yellow color. (4) High increase of proteins indicates symptomatic epilepsy. (5) Concentration of lead in the csp. fluid may be 4x higher than that in the blood.
The above data represent average values.

Malamud, Wm.: The Diagnostic Significance of Cerebrospinal Fluid Examination. J. Iowa State Med. Soc. May, 1934.

Malamud, Wm.: Some Phases of the Biochemistry of the Cerebrospinal Fluid. Med. Record & Annals, Houston, Tex., Aug. 1938.

Merritt, H. H. and Ch. Brenner: Clinical Examination of the Cerebrospinal Fluid. Wisconsin Med. J., March, 1946.

Mestrezat, W.: Le liquide céphalo-rachidien. A Maloine, Paris, 1912.

Roseman, E., M. Rosenbaum, Ch. D. Aring, and E. R. Ferris, Jr.: Cerebrospinal Fluid Dynamics in Man. Arch. Neurol. & Psychiat., 46: 72, 1941.

Sachs., H. Wilkins, and C. F. Sams: Studies on Cerebrospinal Circulation by a New Method. Arch. Neurol. & Psychiat. 23: 130, 1930.

Schmitt, N.: Kolloidreaktione der Rueckenmarksfluessigkeit. Th. Steinkopff, Leipzig, 1932.

Walker, B. S., N. C. Telles, and E. J. Pastore: Amino Acids of the Cerebrospinal Fluid. A.M.A. Arch. Neurol. & Psychiat. 73: 149, 1955.

Wallace, G. B. and B. B. Brodie, B. B.: On the Source of the Cerebrospinal Fluid. The Distribution of Bromide and Iodide throughout the Central Nervous System. J. Pharmacol. & Exper. Ther. Vol. 70, No. 4, 1940.

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THE HISTORY AND CHARACTER OF GOITER IN CENTRAL AMERICA AND MEXICO. ITS SIGNIFICANCE FOR THE ETIOLOGY AND PREVENTION OF THE DISEASE.[a]

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General

In a previous paper, (Greenwald, 49), the history of goiter in the region occupied by one of the great pre-Columbian cultures was examined. It was shown that there is no evidence for the presence of goiter in the Inca Empire until long after the Conquest and that the descriptions of the natives written at that time or shortly thereafter are so complete as to make it certain that goiter was not endemic. The subsequent history of the disease was reviewed and attention was directed to the later great prevalence of goiter in Peru and Argentina, to the remarkable association with deaf-mutism in both countries and to the spontaneous diminution in both goiter and deaf-mutism in Argentina in the latter part of the nineteenth century.

The present paper is concerned with the history of goiter in the home of two other great Indian civilizations, and in adjoining regions, in which territory goiter is very common at present.

As in Peru, it has been claimed that there are ceramic figures of men and women that show goiter. One of these (Mexican) in the Howard Dittrick Museum at the Cleveland Medical Library, appears to be a forgery.*

Under the heading "Bocio exoftalmico," Jost (62) reproduced color photographs of three ceramic figures from three different sites in Mexico. All are to be considered authentic for their inclusion was approved by Dr. Daniel F. Rubin de la Borbolla, Director of the Museo Nacional de Antropologia e Historia. One of these shows exophthalmos but not goiter (this is so stated); two others, a swelling in the neck but no exophthalmos. In one

* This is the opinion of Dr. Gordon F. Eckholm of the American Museum of Natural History, New York. There are many Mexican forgeries of ancient ceramics. A large volume is devoted to them. (Batres, 11).

of these, the swelling is not obvious in the printed photograph. The other portrays a smooth, symmetrical swelling reaching from the chin to the chest. It has a purple color and this color extends to, and includes, the lower part of the nose. To me, identification as goiter appears extremely questionable.

Turning to Guatemala, Dr. John H. Talbott (99) has shown me a color photograph of a figure showing a small, median swelling in the neck that he saw in the museum at Chichicastenango in 1952.

Recently, Borhegyi and Scrimshaw (15), after examining thousands of Guatemalan figurines, published photographs of the only two that they consider to portray goiters. Apparently, they did not know of, or accept, the one seen by Talbott. One shows a small median swelling, apparently at, or just above, the upper end of the sternum, which is rather low for a goiter. The other portrays a greatly deformed face. So far as may be judged from the printed photograph, the supposed goiter may be only an unusually prominent larynx. (I have seen at least two equally prominent among my colleagues).

Even if all of these figures represented goiters, they would not be evidence of the *endemicity* of goiter. Otherwise, we should need to conclude that kyphosis, achondroplasia and two-headed monsters were similarly endemic. Borhegyi and Scrimshaw agree that goiter was not endemic in pre-Hispanic Guatemala and state that their two figurines "might represent sporadic cases of a relatively infrequent condition."

Guatemala and Other Parts of Central America

There are several accounts of the people of this region at, or shortly after, the Conquest. (Alvarado, 3; Diaz del Castillo, 27; Garcia de Palacio, 43; Mendieta, 68; Oviedo y Valdez, 78; Pineda, 83; Remesal, 85; Ximinez, 119). None of these seems to have noted the presence of goiters nor is there any mention of these in the writings of two modern scholars concerned with that period. (Brasseur de Bourbourg, 16; Genet and Chelbatz, 44).

The first record of goiter anywhere in Central America (or Mexico) was given by Gage (41), who saw many, some very large, in Sacapulas, Guatemala in 1628. He was told that these

were due to the water but that he need not fear to drink chocolate for the heating made the water harmless!

It is noteworthy that Gage had previously travelled through a considerable part of Mexico and, after leaving Sacapulas passed through what were later severely affected districts as far south as Cartago and Nicoya, in Costa Rica. Nevertheless, the only goiters mentioned were those at Sacapulas. In particular, he walked through the Valle de las Vacas, where goiter was common by 1690, "especialmente en gente humilde y pobre." (Fuentes y Guzman, 40).

In 1798, Domingo Hidalgo (28) wrote of Jacaltenango: "En este pueblo todos tienen gueguechos, o hinchadas de gargantas, a causa de las males aguas que beben." (In this village, all have "gueguechos" or swellings in the throat, due to the bad water that they drink.) He added a footnote referring to Gage's astonishment at the goiters he had seen at Sacapulas. However, although Domingo described Sacapulas also, he did not mention the presence of goiter there or at any other place but Jacaltenango. It may have been fairly common in many places or it may have disappeared, nearly or completely, from Sacapulas, as has been the case in many other communities. (Greenwald, 47, 48, 52; Hirsch, 60; Osler, 77).

That the disease was really of rather limited distribution, even in the vicinity of Sacapulas is indicated by the fact that a vocabulary of the Mame language used, in that region, published in 1664, had names for fourteen other diseases but none for goiter. (Reynoso, 86). Moreover, there is none in a vocabulary of the Cakchiquel language compiled in 1693 (Thomas de Santo Domingo, 101) nor in one of the Quiché language (Anonymous, 5) compiled at an unknown place but copied, probably at Sacapulas, in 1787. However, the copyist adding his own list of diseases gave a Quiché word for *huehuecho*. This, also spelled *güegüecho*, is the common word for goiter in Guatemala. While the copyist seems to have believed it to be Spanish, it is Indian and also means "turkey." The use of the same word to mean goiter is a variant of the more common practice of denoting goiter by an older word originally meaning the crop of a bird.

We have many reports of goiter in various parts of Central America in the nineteenth century.

In 1825, Thompson (102) disembarked at Acajatlan and went to the neighboring town of Sonsonate, both in San Salvador. He wrote: "The inhabitants of Sonsonate, particularly the Creoles, are dreadfully afflicted with the *goitres*, or as they call them, *buches*." He then went to Guatemala City and later, left the country by the Rio Yzabel (east coast). He made no other mention of goiter.

Only two years later, Dunn (32) almost exactly reversed the route taken by Thompson. He reported: "In some of the provinces the inhabitants are dreadfully afflicted with a swelling in the glands of the throat, vulgarly called 'güegüechos': these sometimes grow to an enormous size, and when accompanied by idioty or extreme imbecility of mind, which is by no means uncommon, they furnish a most humiliating and painful spectacle."

At about this period, Haefkens (55) saw many people with goiter in Guatemala City and noted that most of them were mentally deficient and that many were complete idiots.

A few years later, Montgomery (69) saw many people with goiter at several places in Guatemala and was told that there were villages in which one person in five was so afflicted.

Shortly thereafter, Dunlop (31) found goiter common in all the mountainous parts of Central America. (He visited all of the countries except Honduras.) He stated "I have not seen it so general among all ages and sexes as in Chinameka" (San Salvador).

In 1850 and 1851, Taylor (100) saw many large goiters and several cretins at Medereri and at Masaya, both in western Nicaragua.

Noteworthy in many of these accounts are the frequent references to goiters seen at, or near, the sea. The report by Thompson has already been mentioned. In 1852, Squier (94) wrote: "In Chinandega (Nicaragua, I. G.), as in fact in every other town of the state, I observed numerous instances of the goitre. Chinandega is only seventy feet, and Leon, Granada and Rivas not more than one hundred and fifty feet, above the tide water: yet in all these towns, the goitre is common."

Not long after, Scherzer (91), travelling from Salvador to Sonsonate, made a similar observation at Guaymoco, barely 400 feet above the sea.

In 1864, Bernoulli (12) wrote:

"Die Intensität der kropferzeugende Ursache ist, wie in Europa, in verschiedenen Gegenden sehr verschieden: an die Küsten und in der Tierra templada ist sie im allgemeinen heftiger als auf den Kälteren Hochebenen, es gibt Dörfer, wo sich kaum ein vom Kropf verschontes Individuum findet." (The intensity of the goiterogenic factor is, as in Europe, very different in different regions, in general, it is more severe on the coast and in the moderate zone than on the colder high plateaus. There are villages in which there is scarcely a person without a goiter.)

It is important to remember that these were obvious goiters, not enlargements of the thyroid that are detectable only by bending the head back or by palpation, or both.

Bernoulli continued: "Acute, d.h. in Zeit von wenigen Tagen entshende, bedeutende Kröpfe sind keine Seltenhit." (Acute goiters of noteworthy size that develop in a few days are not unusual.)

Some years earlier, de Escobar (33) had seen and reported what has been observed repeatedly in Europe, and, by writers of reviews and textbooks, even more frequently neglected:

"Next, it is to be remembered that in these settlements, there are few persons without goiter; and that the water in the highlands, so far from engendering this disease, even cures it, while in the valleys below, on the other hand, they produce it."

The idea that goiter is primarily a disease of mountainous countries appears to have led Wells (108) into a curious error. He wrote (p. 547) on Honduras in about 1855: "Goitre is not confined to any particular locality, but is most common in the mountainous districts where, as in Switzerland, the poorer classes are subject to it. I met with but three or four instances of it." Two pages later: "As in Chinandega, Nicaragua, some of the women of Olancho (a low-lying part of Honduras, I. G.), are disfigured by a large swelling, or wen, protruding from the fore-part of the neck developing under the chin. It presents an unseemly sight, and has never been satisfactorily accounted for. It has been mistaken by strangers for the goitre and may be identical with güegüecho, or swelling of the glands of the neck, mentioned by Dunn in his work on Guatemala." Wells was not, unfortunately, the last writer on goiter to allow his preconceived notions to blind him to what he saw.

In 1911, Guerrero (53) believed that the small area (about

five square miles) between Duenas and Acatenango, Guatemala was *the* endemic district and that the nearby capital city was free from the disease. He even had an explanation: the presence of aluminum silicate in the water of the former and its absence in that of the latter.

The earliest report of goiter in Costa Rica appears to be that of its presence in San José in 1853–4. (Wagner and Scherzer, 107). It was not mentioned in their accounts of Cartago and of Puntarenas. (See below). A few years later, Schwalbe (92) stated that goiter was widely distributed through the highlands of Costa Rica. Almost a century later (1943), Picado (82) wrote that goiter was so common in San José that the inhabitants were known as "guëchos."

Apparently, San José has lost its sad preeminence. Perez and associates (81) recently made a survey of 26,768 individuals, about 11 per cent of all the population of Costa Rica from 7 to 18 years of age. San José had only 12 per cent of goiters, whereas Puntarenas and Guanacaste, both on the coast, had 20.3 and 25.6 per cent, respectively.

Hyperthyroidism appears to have been rather infrequent in Costa Rica. Of 6000 patients at the Hospital de San Juan de Dios, at San José, which received patients from the entire country, only one was diagnosed as "sindrome de Basedow" and this, at autopsy, proved to be carcinoma. (Garcia Carrillo, 42). In 1944, in the Canton of Puriscal, the southwest corner of the state of San José, Ureuyo Gallegos (103) found one case of hyperthyroidism among 25 adults and one child with goiter.

Mexico

Neuburger (73) included goiter among the diseases recognized and treated by the ancient Mexicans. He gave no authority for this but stated (footnote, p. 40) that his paper had been based on the work by Flores (38).

That author wrote, under the heading "Laringitis" (Vol. 1, p. 129):

"Entre los afecciones de los anexos de la larginge parece que conocieron el bosio quechpozauiliztli o tozcapuzauliztli de que hacen mencion los autores que se ocupan de su antiqua historia." (Among the diseases of the parts adherent to the larynx appears

to be that which we call goiter quechpozauiliztli or tozcapuliztli as has been mentioned by authors concerned with its history). He gave no authority for this statement and I know of no one other than myself who has written on the early history of goiter in Mexico.

Since Flores, there have been several whose writings give evidence of consultation of original sources: Gerste (45); Rodriguez (87); Stecker (98); Deuchler (26). None of these mentioned goiter.

Even more than for Guatemala, we have abundant and detailed descriptions of Mexican life at and shortly after the Conquest. Some of these were written by companions of Cortez. Of this group, the best is that by Diaz del Castillo (27). Blanton (13) examined this for medical references. Neither he nor I found any mention of goiter.

Our most complete account is given by Sahagun (89). He came to Mexico in 1529, only seven years after the Conquest. He became interested in the life, customs and knowledge of the Mexicans and began to write his book in Aztec about 1546. Later, he prepared a Spanish text. Various editions and translations have appeared but the most satisfactory is a complete reproduction with all of the hundreds of illustrations that was published from 1905 to 1938. (89).

In that we may read Vol. 3, p. 98):

"Para la enfermedad de las paperas, e hinchabones de la garganta, sera necesario frotar con le mano la garganta con cierte hierba llamada *cocozihuite* mezelando con cisco de la olla, y beber el agua de la hierba llamada *ahacaxilotic*." (For the disease "paperas" and swellings of the throat, it was necessary to rub the throat, by hand, with a certain herb called *cocozihuite* mixed with soot from a pot (?) and to drink an infusion of the herb called *ahacaxilotic*.)

In another paper (50), I have discussed the various and changing meanings of "paperas." Originally, it seems to have been applied to any swelling in the neck, apparent on the exterior. Scrofula (lamparones) seems to have been the first to have been distinguished from others. For a long time, "paperas" included both mumps and goiter: now, it means only the former.

That it did *not* mean goiter in Mexico in the sixteenth century

is shown by three instances of its use to designate an epidemic disease that was fatal to many. Thus, in the Anales de Tlatelolco, we find an entry for 1549: "Cuando hubo paperas, por lo que murieron muchas gente."

The Spanish text of the Codex Telleriano-Remensis (Hamy, 56), which was completed before 1562, has a passage: "Ano de seis conejos y de 1550 murieron yndios en esta Nueva Espana de paperas." (In the year of six rabbits, or 1550, many Indians in New Spain died of "paperas.") Kingsborough (64) translated "paperas" as "measles." Stecker (98) believed it to have been either mumps or diphtheria. Whatever it may have been, it was certainly not goiter that killed many Indians in 1550.

Mendiete (68) wrote: "En fin del ano de noventa y cinco y en trado el de noventa y deis, al tiempo yo esta escribia, vino otra generalisma pestilencia, nezclada de serampion, paperas y tabardillo." (At the end of the year (15)95 and beginning of the year (15)96, when this is written, there came another general pestilences, a mixture of measles, "paperas" and scarlet fever). The conjunction with measles and scarlet fever is a clear indication the "paperas" did not mean goiter.

In 1577, in reply to a long official questionnaire, the authorities of Ameca, in Jalisco, gave a detailed reply to a question as to what diseases were prevalent and what was done for them.

"Sarna, paperas y bubos" were said to be treated with a "trementina" (turpentine) made from a certain tree and a certain herb. The association of "paperas" with scabies and syphilis and the manner of treatment afford additional evidence that "paperas" did not mean goiter. (Amaya, 4).

There are many similar "relaciones," some in reply to the same questionnaire and others to those of later periods, down to the end of the eighteenth century. Many of these have been published by Paso y Troncoso (79) and some by others. I have not found any indication of goiter in any of these.

In 1584, Alonso Ponce (Alonso de San Juan) began his travels of 2557 "leguas" (8520 miles). His account was published in 1872 (84) and indexed by Guerrero (54) in 1949. Only three diseases were mentioned: (1) cocolitzle, a pestilence that killed many and wiped out small settlements; (2) syphilis and (3) lamparones (scrofula), which the Indians called "Castellan zab"

or "bubas de Castilla" (Castillian swellings) because they had not known them before the Spaniards came.

We also have the description of northern Mexico of about 1603 by de la Mota y Escobar (70) and another of 1621 by Arregui (7). Both of these mention the physique and the diseases of the Indians, but I have found no mention of goiter in either.

We have several accounts of the "superstitions" of the Indians: Cardenas (20), Serna (93), Ruiz de Alarcon (88), Balsalabre (10), dating from 1591 to 1656. The treatment of "garganta hinchada" (swollen throat) is described but there is no mention of endemicity nor is there any other indication of the existence of goiter.

Several books of occidental medicine appeared in Mexico during the colonial period. Thus, in 1579, Farfan (35) published a "Tractado breve de anathomia y chirurgia y de algunas enfermedades que mas comunmente suelen haver en esta Nueva Espana." Bocio (goiter) was considered at length but, in spite of the title, there was very little to indicate that Farfan had any experience with goiter and none at all to show that he knew it to be endemic anywhere in Mexico.

A few years later, in another book by Farfan "Tractado breve de medicina" (36), there is no mention of "bocio," nor even of "paperas."

In 1672, Lopez (66) published in Mexico, his "Tesoro de medicinas para toda enfermedades." The third edition, of 1708, has a recipe for an ointment for "paperas" but there is no mention of "bocio."

About forty years after Lopez, Esteynefer (34) compiled a book on medicine for the use of Jesuit missionaries in Mexico and California. "Parotidas" was mentioned but neither "paperas" nor "bocio."

Even late in the eighteenth century, Venagas (106) under the caption "Tumores" wrote:

"Los ganglios son unos tumores duros, comunmente indolente, que no mudon el color de la piel, situado de ordinario en el carpo, tarso, rodella o flexura del codo. Nacen de la expansion, o abolsamente de la glandulas. De este origen son tambien los bocios" (Ganglions are hard tumors, generally indolent, which do not change the color of the skin, and, ordinarily, are situated in the

wrist, ankle, knee or elbow. They arise by the growth, or budding, of the glands. Goiters also have this origin). In spite of the title of the book, it may well be doubted that Venegas had seen many goiters, if any at all.

In 1945, I (47) believed that Chappé d'Auteroche had seen goitrous women at Las Bigas or Las Vigas, near Jalapa, in 1769. Later examination of the original French (22): "Les habitants de los Bigas sont mulatres; les femmes y vont a moitié nues, & laissent voir la plus vilaine gorge due monde" has led me to believe that the women had the ugliest breast(s) in the world.

The first clear record of goiter in Mexico appears to be that of Thompson (102), who, in 1825, at Tierra Colorado, near Acapulco, saw three goitrous sisters, one of whom appeared to be a cretin. The first record of *endemicity* is that of Hardy (57), who saw many goiters in several places in Sonora between 1825 and 1829.

After seven years residence and travel in Mexico, with, as he claimed, extensive reading of the accounts of others, Mühlenpfordt (71) wrote of the excellent physique of the Mexicans. Goiter was mentioned only incidentally and only in Michoacan. "In den Küstengegenden und am unteren Theile der westlichen Abhänge der Cordilleren sind während eines grossen Theile des Jahres Wechselfieber, hie und da auch Faulfieber häufig. Kröpfe send ebenfalls ein gewöhnliches Ubel hier. . ." (In the coastal regions and on the lower part of the western slopes of the Cordillera, malaria and, here and there, typhus, are common during a great part of the year. Goiter is also usual here. . .

At about the same time, Gilliam (46) found that half of the population of Canales in Durango had goiters, many of them large enough to interfere with speech and respiration and some so heavy as to require suspension in a sling. Cretinism was also common.

A few years later (1856), Wilson (109), at Tierra Colorado (see Thomson, above) wrote: Here prevails not only that harmless cataneous affection, the *Quiricua*, which causes people to appear spotted or painted (Pintos), but also *Cretinism*, the much more formidable disease so prevalent among the mountains of Switzerland.

In 1848 (Heller, 58) and again in 1863 (Leon, 65) and in 1865 (Coindet, 24), there were reports of goiter as endemic in

parts of Tabasco. Heller also described the neighboring state of Chiapas (59) but made no mention of goiter there and Leon expressly stated that there was little goiter in the mountainous districts of that state. Both Leon and Coindet emphasized that there were no cretins in Tabasco.

Coindet (25) stated that goiter was common in other districts of low altitude but was rare on the high plateau, although several cases had been seen, in 1862, at San Andres, at the foot of Mt. Orizaba. The same statement as to the rarity of goiter on the high plateau was made by Jourdanet (63).

By 1884, goiter had become sufficiently common in many parts of Mexico to warrant its inclusion in an inquiry made by the Secretario del Fomento in preparation for a medical geography of Mexico. The information collected was published in two forms: (1) a book by Velasco (105) and (2) two volumes, one of maps and the other of comment, by Orvañanos (76). The latter author gave the more detailed information. The goiter map was colored to show "distritos" in which no "municipalidad" reported goiter, those in which from 3 to 5 percent had goiters, and those in which goiter was present in every "municipalidad." Proceeding from the south, the most affected "distritos" were Tuxtlan in Vera Cruz (on the coast); Alvarez, Mina and (coastal) Union in Guerrero; Huetamo in Michoacan; Huejutla in Hidalgo; Jalpan in Queretaro and San Ignacio (on the coast) in Jalisco. As judged by the proportion of total area reporting the presence of goiter, the most severely affected states were Sinaloa, Vera Cruz and Guerrero. Of the four divisions of the Distrito Federal, only one, Tlalpan, reported goiters. None were reported from the states of Aguascalientes, Campeche, Coahuila, Guanajuato, Tamaulipas, Tlaxcala nor from the territories of Baja California and Tepic.

Tabasco had goiters only in districts adjoining the states of Vera Cruz and Chiapas and none in Tabasco reported deaf-mutes or idiots. Closer agreement of the map showing the prevalence of goiter with that of "Tardamudos, Sordo-mudos e Idiotas" was shown in Sinaloa and in some adjoining parts of Chihuahua and Durango.

A recent diminution in the prevalence of goiter was said to have occurred in Sonora and Jalisco.

Of San Bartolomé, in Chiapas, it was reported that goiter was

common in both sexes of the "ladino" (European, or Spanish-speaking) population but only among the women among the "indigena" (Indian). The freedom of the male Indians was ascribed to the fact that they lived for most of the week on the land that they tilled, thus using very little of the town-water.

Some twelve years later, Starr (97) was told that goiter was not so frequent in San Bartolomé as it had been forty years earlier, and that the change had followed the introduction of a new supply of water. (This is not, necessarily, a contradiction to the statement in the preceding paragraph). However, there still were many. The population of 2,000 showed 200 obvious goiters, 50 of them of "notable" size. (For conditions about thirty years later, see Huerta Sanchez, below). On that visit and on two subsequent ones, Starr saw many goiters, some very large, at other places in Chiapas and in Michoacan. He found that they were accompanied by imbecility and deaf-mutism.

In 1920, the results of another survey were presented by Castillo Najera (21). There were several significant differences between this and that of Orvañanos. Guerrero was considered now to be the most severely affected state, with Vera Cruz second and followed by Michoacan and Oaxaca. Goiter was, apparently, much more frequent in Chihuahua than it had been. Of Sinaloa, Castillo Najera stated that the proportion affected by goiter had been diminishing for thirty years. Similar diminutions, not so general, were reported from several places in Oaxaca, Guerrero and Jalisco. The presence of goiter in the Federal District, particularly in Tlalpan, was explicitly denied. (Compare, Orvananos above and Stacpoole, below).

By 1931, goiter was present in Baja California, Coahuila, Guanajuato, Tamaulipas and Tlaxcala, from none of which had it been reported fifty years earlier. (Huerta Sanchez 61, Fernandez Ferio, 37).

Huerta Sanchez also mentioned a number of places in which there had been a great diminution in the prevalence of goiter. On the other hand, San Bartolomé, in Chiapas, in spite of the new water supply mentioned by Starr, still had a great number of goiters—a large majority of the population according to one of the two reporters. That is very much more than the ten per cent seen by Starr.

Another informant reported to Huerta Sanchez that there were

27 cases of goiter among the 1500 inhabitants of San Bartolo, D.F. but there was no other report of goiter in the Federal District.

By 1937, the picture had changed very markedly. Stacpoole (95) reported a great deal of goiter in the Federal District. San Bartolo now had from 31 to 50 percent of goiters. In the southeast, the portion shown by Orvañanos as having goiters in 1884, there were now many villages with from 71 to 70 percent of goiter. The village of Tlalpan, which gave its name to the section, had from 31 to 50 percent.

Had goiter really disappeared from this district (see Castillo Najera's explicit denial of its existence) only to reappear within 17 years? This had occurred in Edmonton, Alberta over a somewhat longer period (Greenwald, 47). Spontaneous diminution and disappearance have been recorded from many places (Blechmann 14; Greenwald, 47, 48; Hirsch, 60; Osler, 77) but reappearance has not been noted nearly as frequently.

In 1953, Stacpoole (96) reported figures for the incidence of goiter in eight states. Tlaxcala, which had been said to be free from the disease in 1884 (Orvañanos) and to have had but few cases in 1930 (Huerta Sanchez, Fernandez Ferio) now had 37 percent, next to the highest proportion (46 percent in Morelos). Guerrero had only 27 percent.

While the association of goiter in Mexico with cretinism was, and is, well-marked, that with deaf-mutism is not at all clear. It was denied by Vazquez Gomez (104). In the absence of statistics for the number of goiters, it is difficult to be certain. However, according to the census of 1895 (Penafiel, 80), the highest numbers of deaf-mutes, per 100,000 population, were in Guerrero, 163; Chiapas, 133; and Sinaloa, 109; all states with a great deal of goiter. However, other states with, presumably, a large proportion of goiters, had comparatively few deaf-mutes: Durango, 58; Morelos, 58; Oaxaca, 69; Michoacan, 75. The lowest proportion, 6 per 100,000, was reported from Tabasco.

I have found comparatively few references to exophthalmic goiter or to other forms of thyrotoxicosis. In 1884, the former was the subject of a thesis by Chavez (23), who described two patients, one from Mexico (city) and the other from Morelia. The latter had been discussed previously by Berruero in his inaugural lecture. Chavez did not mention any other report of this disorder

in Mexico. Neither did he state that his two cases were examples of a rare disease.

In 1897, Augustin M. Dominguez (29) wrote that simple goiter, which had been the predominant disease in the city of Oaxaca had nearly disappeared.* He ascribed the change to the use of sea-salt. He also stated that the remaining cases were of Basedow's disease.

That thyrotoxicosis of any kind was probably infrequent in most of Mexico as late as 1909 may be inferred from the fact that Dominguez (30), in a series of lectures on therapeutics, discussed parenchymatous goiter, cystic goiter and endemic goiter but made no mention of toxic goiter.

By 1930, exophthalmic goiter was reported to Huerta Sanchez (61) from several places. In one, in Jalisco, there were four cases in a family of six persons—with no other goiters in a population of 2000. The great majority of the people in San Bartolomé in Chiapas were said to have exophthalmic goiter.

In 1951, Calzado Lopez (19) reported nine cases of thyrotoxicosis, "seleccianados entre los numerosos que fueron tratados en el Hospital General de Hermosillo, Son." (Interestingly enough, he also mentioned that goiter in Sonora was most common along the Sonora and Sahuaripa rivers. The map published by Huerta Sanchez (61) showed goiter along the latter but not on the former.

In the same year (1951), Burcia Astorga (17) reported that two percent of all the people with goiters in a village on the western edge of Chihuahua had Basedow's disease.

Two other theses have dealt with thyrotoxicosis and exophthalmic goiter (Alpuche Morales, 2; Samano Vidales, 90) but neither of these gives any information regarding the prevalence of the disorder. Apparently, the subject is of interest. This would seem to indicate an increased prevalence as compared with fifty years ago.

It is clear, I believe, that goiter was not endemic in Mexico or in Central America before the Spaniards came, that it appeared first in certain few places and, much later, became general. In many places, there has been spontaneous diminution and, in

* An anonymous Englishman (6) wrote in 1827: "I am inclined to believe that the Indian women of Oaxaca may merit their Reputation, as the Circassions of New Spain." Again, apparently, a cycle of no, or but few, goiters to 20 percent to few, in a period of no more than seventy years.

several, nearly complete disappearance. In the Federal District, from which we should have fairly good information, at least for the last century, it would seem that the disease disappeared only to return to occupy a rather greater area.

Goiter in these countries is undoubtedly a real problem, though one may be sceptical of the significance of some of the later data obtained in children and adolescents by careful examination of the neck.

What Shall Be Done?

For several years, there has been a determined campaign by Stacpoole and by Scrimshaw and associates to secure the iodinization of all the kitchen and table salt used in these countries. The concentration sought is 100 parts of iodine (as potassium iodate) per million of salt.

Borhegyi and Scrimshaw (15) admit that goiter was not endemic in pre-Hispanic Guatemala and that the first record of its appearance is that of Gage. They ascribe this freedom from goiter in the people of the highlands to a hypothetical provision of sefood which supplied sufficient iodine. They rightly dismiss sea salt as an important contributor. But they seem not to have considered how great such commerce would have been required to be nor how it would have been carried without beasts of burden. Nor do they pay any attention to the fact that goiter has been endemic in many places at the sea (see above) and even on quite small islands (Greenwald 47, 51). Even the reports by Perez, Salazar-Baldiocedas, Tandon and Scrimshaw (81) and by Munoz, Perez and Scrimshaw (72) established this coastal goiter for Costa Rica and for Guatemala and Panama.

In 1953, Cabezas, Pineda and Scrimshaw (18) stated that the average iodine content of 22 samples of salt purchased in the markets of El Salvador was 22 parts per million. At least one contained as much as 57. After I (48) had pointed out that these quantities were far greater than the 5 parts per million that had been credited with such marvellous results in Poland and Switzerland, the method of analysis was changed, from one of the two alternate methods of the Association of Official Agricultural Chemists to the other. The difference between the two is in the manner of removing the excess of bromine used to oxidize the iodine to iodate. None of the earlier samples was available for

analysis by the second method (Arroyave, 8) nor is there any evidence that *any* sample of salt was analyzed by the two methods. The second report (Arroyave, Pineda and Scrimshaw, 9) made no mention of the earlier one, only of "datos ineditos."

Only *five* samples of Salvadorean salt were analyzed by this method. They were found to contain from 3 to 8, average 6, parts per million, which is more than the 5 parts that the Swiss iodized salt was supposed to contain and much more than was actually present. To quote from Nicod (74): "It thus appears that the rate of iodization (5 mg of potassium iodide per kg of salt) at present recommended is not always maintained, and since this does not seem to have modified the efficacy of the measure, it may be concluded that the optimal iodine intake is not necessarily as high as has been believed."

As little as ten grams of the salt now recommended furnishes one milligram of iodine from this source alone. This exceeds the customary intake, from all sources other than iodized salt, in any part of the world. (Greenwald, 48). It is not without hazard.

After a very critical examination of the reports of so-called "Jod-Basedow," Flück (39) conceded that 18 cases of Basedow's disease in Switzerland *from 1922 to 1924* might have been due to the use of iodized salt (less than 5p.p.m., see Nicod, above). He regarded larger amounts, but still less than one milligram per day, as likely to be harmful and believed that two milligrams were almost certain to be so.

Even Marine (67), who is certainly not prejudiced against the use of iodized salt, warned, as recently as 1954:

"Since the dangers of Jod-Basedow are serious in endemic goiter regions with a high incidence of advanced adenomatous goiter, it would seem advisable to use (as Switzerland has) a conservative supplement (1:100,000) rather than greater concentrations."

Guatemala, at least, would seem to be in this dangerous position. Of 4475 persons more than 18 years old, 24.1 percent had adenomatous goiters. Of these, more than a fourth, or 6.9 percent of the total, were visible at a distance of several meters, with the head in normal position. (Munoz, Perez and Scrimshaw, 72). It would seem that a great increase in the number of cases of thyrotoxicosis is to be expected.

Munoz, Perez and Scrimshaw (72) wrote (P. 968):

"Since the iodine content of the local salt is so nearly that believed to prevent goiter under ordinary circumstances, it is probable that one or more goitrogenic factors are increasing the physiological need for iodine. . . . . A search must be made for specific goitrogenic factors in the Guatemalan environment."

The present author is in hearty agreement with the last sentence. He would, however, not limit the search for a "specific, goitrogenic factor" to the Guatemalan environment. He believes that the history of goiter in Central America and in Mexico indicates that the "specific, goitrogenic factor" in these countries, as elsewhere, is some kind of a biological agent, absent in the pre-Hispanic period and introduced into Guatemala some years after the Conquest but, apparently, not into Mexico for about three centuries thereafter.

Summary

The early literature descriptive of the inhabitants of Central America and Mexico show that goiter was probably not known and certainly was not endemic before the Spaniards came. It was first reported from but one place in Guatemala early in the seventeenth century and, somewhat later, in various other places. It later became general in Guatemala and other Central American countries. The earliest reports of such general prevalence are of the nineteenth century. At this same late date, are the first reports of the presence of goiter anywhere in Mexico.

Throughout the region, goiter was originally more prevalent along the coast and in the lowlands than on the high plateaux. It is still common in many coastal areas.

Association with cretinism and, to a lesser degree, with deaf-mutism was noted quite early. Thyrotoxicosis of any kind seems to have been, and probably still is, comparatively infrequent.

Sopntaneous diminution in prevalence has been recorded from several places.

It is believed that the history of goiter in this region, as elsewhere, is that of an infectious disease.

It is believed that the use of salt containing 100 parts of iodine per million may be fraught with undesirable consequences.

BIBLIOGRAPHY AND NOTES

1. Aguirre, P. translator. Anales de Tlatelolco, 1519–1630. Mexico. 1950.
2. Alpuche Morales, E.: El sindrome de Parry Graves Basedow. Thesis. Mexico. 1953.
3. Alvarado, P. de, translated by S. J. Mackie: An Account of the Conquest of Guatemala in 1524. New York. 1924.
4. Amaya, J.: Ameca. Protofundia Mexicana. Historia de la propriedad del valle de Ameca, Jalisco y circumvecinidad. Mexico. 1951.
5. Anonymous. Spanish-Quiché Vocabulary. Manuscript. Peabody Museum, Harvard Univ. Photocopy in New York Public Library.
6. Anonymous. Diary of Travel from Mexico to Mining Districts, 1826–7. Manuscript. Library of The Hispanic Society, New York.
7. Arregui, D. L. de: Descripcion de Nueva Galicia. (c.1621) Sevilla. 1946.
8. Arroyave, G.: Letter of December 2, 1958.
9. Arroyave, G., O. Pineda, and N. S. Scrimshaw: Contenido de yodo de muestras de sal comun provenientes de Centro America y Panama. Bol. Oficina Sanit. Pan-Americana, 42, 586–588, 1957.
10. Balsalobre, G. de: Relacion autentica de la idolatrias supersticiones vanas observaciones de los indios del opispada de Oaxaca. An. museo nac. Mexico, 1 epoca, 6, 225–260, 1892.
11. Batres, L.: Antigüedades Mejicanos falsificados. Falsificacion y falsificadores. Mexico. 1910.
12. Bernoulli, G.: Bemerkungen uber Tropenkrankeiten, Schweiz. Zeitschr. Heilk. 3, 100–139, 1864.
13. Blanton, W. B.: Medical References in Bernal Diaz's Account of the Discovery and Conquest of Mexico. Ann. Med. Hist., 3rd Ser., 4, 399–405, 1942.
14. Blechmann, C. Le goitre en France. Le Concours Medical, 14, 1035–1039, 1934.
15. Borhegyi, S. F., and N. S. Scrimshaw: Evidence for Pre-Columbian Goiter in Guatemala. Amer. Antiquity, 23, 174–176, 1957.
16. Brasseur de Bourbourg, M. l'Abbe: Histoire des nations civilisées du Mexique et de l'Amerique Centrale durant les siecles antérieurs a Christophe Colomb. 4 vols. Paris. 1857–1859.
17. Burcia Astorga, J. R.: Bocio simple ye tirotoxicosis e informe general sobre la exploracion sanitaria de la villa de Chinipas, Distrito Arteago, Estado de Chihuahua. Thesis. Mexico. 1951.
18. Cabezas, A., T. Pineda, and N. S. Scrimshaw: Endemic Goiter in El Salvador School-Children. Amer. J. Publ. Health, 43, 265–268, 1953.
19. Calzado Lopez, F. Tirotoxicosis. Thesis. Mexico. 1951.
20. Cardenas, J. de: Primera parte de los problemas y secretos maravillos de los Indias. Mexico. 1591. Reprint Madrid. 1945.
21. Castillo Najera, F.: Contribucion al estudio del bocio endemico en la Republica Mexicana. Revista mex. biologia, 1, 47–60, 1920.
22. Chappé d'Auteroche: Voyage en Californie pour l'observation du passage de Venus sur le disque du soleil le 3 Juin 1769 . . . . Paris. 1772.
23. Chavez y Aparicio, L.: Brevismo estudio sobre el mal de Basedow. Thesis. Mexico. 1884.
24. Coindet, L.: Extracts from a letter of . . . . Recueil Mem. Med. Chir. Pharm. Militaires. Series 3. 13, 162–172, 1865.

25. Coindet, L.: Le Mexique considerée au point de vue medico-chirurgical. 3 vols. Paris. 1863.
26. Deuchler, W.: Juan de Cardenas. Ein Beitrag zur Geschichte der Spanischen Naturbetrachtung während des 16ten Jahrhunderts. Bern and Leipzig, 1930.
27. Diaz del Castillo, B.: translated by A. P. Maudsley. The Discovery and Conquest of Mexico. 1517–1521. New York. 1956.
28. Domingo Hidalgo. J. Descripcion de la provincia de Totonicapan. Gazeta de Guatemala, 2, 169–202, 1798.
29. Dominguez, A. M.: Contribuciones medicas de la ciudad de Oaxaca. Thesis. Oaxaca. 1897.
30. Dominguez, M.: Breves apuntes de terapeutica. La Escuela de Medicina. (Mexico) 24, 110–111, 121–122, 1909.
31. Dunlop, R. G.: Travels in Central America . . . . New York. 1847.
32. Dunn. H.: Guatimala. or the United Provinces of Central America in 1827–28. New York. 1828.
33. Escobar, A. de: Account of the Province of Vera Paz in Guatemala and of the Indian Settlements or Pueblos Established Therein. J. Royal Geog. Soc., 11, 89–97, 1841.
34. Esteynefer, J. de: Florilegio medicinas de todas las enfermedades. Mexico. 1713.
35. Farfan, A.: Tractado breve de anothomia y chirurgia y de algunas enfermedades que mas communmente suelen haver en esta Nueva Espana. Mexico. 1579.
36. Farfan, A.: Tractado breve de medicina. Mexico. 1952. Facsimile reprint. Madrid. 1944.
37. Fernadez Ferio, D., and U. Marcelos del Raso: El bocio en Mexico. Mexico. 1933.
38. Flores, F. A.: Historia de la medicina in Mexico, 3 vols. Mexico. 1886.
39. Fluck, W.: Die Schweizerische Basedowstatistik von 1922–24. Schweiz. med Wochschr., 9, 28–36, 1928.
40. Fuentes y Guzman, F. A. de: Historia de Guatemala o Recordacion florida escrita en sigle XVII . . . . 1690. Reprint 2 vols. Madrid. 1882–3.
41. Gage, T.: The English-American his travail by sea and land: or a new survey of the West Indies. London. 1698.
42. Garcia Carrillo, E.: Datos Epidemiologicas sobre el Bocio y sobre la Fiebre Reumatico en Costa Rica. Revista medica (San José, Costa Rica) 4, 558–561,1941.
43. Garcia de Palacio, D.: Carta dirigida al Rey de Espana. 1576. In Collection of rare and original documents and relations concerning the discovery and conquest of America. No. 1. ed. E. G. Squier. New York. 1860.
44. Genet, J., and P. Chelbatz: Histoire des peuples mayas-quichés. Paris. 1927.
45. Gerste, A.: Notes sur la medécine et la botanique des anciens Mexicains. Rome. 1909.
46. Gilliam, A. M.: Travels over the Table-Lands and Cordilleras of Mexico during the Years 1843 and 1844. Philadelphia. 1846.
47. Greenwald, I.: The Early History of Goiter in the Americas, in New Zealand and in England. Bull. Hist. Med., 17, 229–268, 1945.

48. Greenwald, I.: The Human Requirement for Iodine. Amer. J. Clin. Nutrition. 3, 215–224, 1955.

49. Greenwald, I.: The History of Goiter in the Inca Empire: Peru, Chile and the Argentine Republic. Texas Reports on Biology and Medicine. 15, 874–879.1957.

50. Greenwald, I.: Notes on the History of Goiter in Spain and among Jews. Bull. Hist. Med., 32, 121–136, 1958.

51. Greenwald, I.: Goiter in Japan. Trop. Geog. Med., 10, 149–153, 1958.

52. Greenwald, I.: Iodine Prophylaxis of Goitre. Lancet 1958I. 1071.

53. Guerrero, P.: Investigaciones sobre etiologica del bocio endemoco. La Juventud Medica, 11 (3) 1–5, 1911.

54. Guerrero, R.: Indice clasificado de la relacion breve verdedera de algunas cosas de las muchas que sucedieron al Padre Fray Alonso en las provincias de la Nueva Espana. Mexico. 1949.

55. Haefkens, J.: Centraal Amerika. Dordrecht. 1832.

56. Hamy, E. T.: Codex Telleriana-Remensis. Paris. 1899.

57. Hardy, R. W. H.: Travels in the Interior of Mexico in 1825, 1826, 1827 and 1828. London. 1829.

58. Heller, C.: Veber den Staat Tabasco. Sitzungsberichte. Mathematisch-Naturwissenschaftliche Classe. Akademie der Wissenschaften, Wien. 1 (3) 117–124, 1848.

59. Heller, C. Ueber den Staat von Chiapas und Soconusco in der Republik Mexico. Ibid., 124–136.

60. Hirsch, A. translated by C. Creighton.: Handbook of Geographical and Historical Pathology. 3 vols. London. 1885. Vol 2, 121–202.

61. Huerta Sanchez, L.: Bocio y su distribucion geografica en la Republica Mexicana. Thesis. Mexico. 1931.

62. Jost, M. Medicina Precortesiana. Mexico. 1952.

63. Jourdanet, D. Influence de la pression de l'air sur la vie de la homme. 2 vols. Paris. 1875.

64. Kingsborough, E. K.: Antiquities of Mexico. 9 vols. London. 1831–1848. Vol 6, p. 151.

65. Leon, J. J.: El bocio endemico de Tabasco. Bol. soc. mex. geog. y estadistica, 10, 345–363, 1863.

66. Lopez, G.: Tesoro de medicinas para todas enfermedades. Mexico. 1672.

67. Marine, D.: Endemic Goiter. A Problem in Preventive Medicine. Ann. Internal Med., 41, 875–886, 1954.

68. Mendieta, G.: Historia eclesiastica indiana. Mexico. 1870.

69. Montgomery, G. W.: Narrative of a Journey to Guatemala in Central America in 1838. New York. 1839.

70. Mota y Escobar, A. de: Descripcion geografica de los reinos Nueva Galicia, Nueve Vizcaya y Nueva Leon. 2nd. Mexico. 1940.

71. Mühlenpfordt, E.: Versuch einen getreuen Schilderung der Republik Mejico. 2 vols. Hannover. 1844.

72. Munoz, J. A., C. Perez, and N. S. Scrimshaw: Endemic Goiter in Guatemala. Am. J. Trop. Med. Hyg., 4, 963–969, 1955.

73. Neuburger, M.: The Medicine of the Ancient Mexicans. In Essays on the History of Medicine. New York. 1930.

74. Nicod, J. L.: Le goitre endémique en Suisse et sa prophylaxe par le sel iodé. Bull. World Health Org., 9, 259–273, 1953.

75. Ocaranza, F.: Historia de la medicina en Mexico. Mexico. 1934.

76. Orvañanos, D.: Ensayo de geografia y climatologia. 2 vols. Mexico. 1889.

77. Osler, W.: On Sporadic Cretinism in America. Am. J. Med. Sciences, 106, 506–518, 1893.

78. Oviedo y Valdez, G. F. de: Histoire de Nicaragua. In Ternaux-Compans, H.: Voyages, relations et memoires . . . . Vol. 14. Paris. 1840.

79. Paso y Troncoso, F. del: Papeles de Nueva Espana. 4 vols. Mexico. 1944–1947. Ibid. 9 vols. Mexico. 1952.

80. Penafiel, A.: Cuadro synoptico y estadistico de le Republica Mexicana. Bol. soc. Mex. geog. y estadistica, 5 epoca, 1, 51–132, 1902.

81. Perez, C., A. Salazar-Baldiocedas, O. B. Tandon, and N. S. Scrimshaw: Endemic Goiter in Costa Rican School Children. Am. J. Public Health, 46, 1283–1286, 1956.

82. Picada, T. C. Investigaciones sobre fisiopatologia tiroidea. San José (Costa Rica). 1943.

83. Pineda, J. de: Descripcion de la provincia de Guatemala. Ano 1594. In Colleccion de libros y documentos referentes a la historia de America. Vol. 8. Madrid. 1908.

84. Ponce, A. (Alonso de San Juan).: Relacion breve verdadera de algunas cosas de las muchas que sucedieron al Padre Fray Alonso Ponce en la provincias de Nueva Espana. Madrid. 1872.

85. Remesal, A. de: Historia general de las indias occidentales y particular de la gobernaccion de Chiapas y Guatemala. Madrid. 1619. Reprint. Guatemala. 1932.

86. Reynoso, D. de: Vocabalario de la lengua mame . . . . 1664 Reprint. Mexico. 1916.

87. Rodriguez, L. A.: La ciencias medicas de los Aztecas. Mexico. 1916.

88. Ruiz de Alarcon, H.: Tratado de las supersticiones y costumbres gentilicas que yo viven entre los indios naturales desta Nueva Espana . . . . escrito en Mexico, ano 1629. An. Museo nac. Mexico, 1 epoca, 6, 125–223, 1892.

89. Sahagun B. de: Historia general de las cosas de Nueva Espana. 8 vols. Mexico. 1905–1938.

90. Samano Vidales, G.: Tirotoxicosis. Thesis. Mexico. 1953.

91. Scherzer, C.: Travels in the free states of Central America: Nicaragua, Honduras and San Salvador. 2 vols. London. 1857.

92. Schwalbe, C.: Klima and Krankheiten der Republik Costa Rica. Deutsche Archiv Klin. Med., 15, 133–172, 318–349, 1875.

93. Serna, J. del: Ministros de indias para el conocimiento de sus idolatrias, y extirpacion de ellas, escrita en 1656. An. Museum. nac. Mexico, 1 epoca, 6, 265–487, 1892.

94. Squier, E. G.: Nicaragua: Its people, scenery and monuments. 2 vols. New York, 1852.

95. Stacpoole, H. H.: Bocio Endemico en el Distrito Federal y Estudio comparativo de la Inteligencia de los Ninos Bociosos e Indemnes de esta Entidad. Trans. Third. Internat. Goiter Conference and Am. Assoc. Study of Goiter. 1938.

96. Stacpoole, H. H.: Prophylaxis of Endemic Goiter in Mexico. Bull. World Health Org., 9, 283–291, 1953.

97. Starr, F.: In Indian Mexico. Chicago. 1908.

98. Stecker, G.: Krankheiten in Mittel Amerika zur Zeit des Columbus, Janus, 28, 232–301, 1924.

99. Talbott, J. H.: Personal communication.

100. Taylor, W. Report on Goitre. Trans. Med. Assoc. State of Alabama. 7, 78, 1854.

101. Thomas de Santo Domingo. Vocabulario de la lengua Cakchiquel 1693. Manuscript. Bibliothéque national, Paris. Photocopy in the Library of the Hispanic Society, New York.

102. Thompson, G. A.: Narrative of an Official Visit to Guatemala from Mexico. London. 1829.

103. Ureuyo Gallegos, C.: Investigaciones preliminares sobre Distrofias Tiroideas en el Canton de Puriscal. Revista medica (San José, Costa Rica) 5, 181–184, 1944.

104. Vasquez-Gomez, F.: Causas mas frecuentes de la sordo-mudez en Mexico y resultados de la ensenanza de la languaje articulado en la escuela N. de sordo-mudez. Revista di anatomia patologica y clinicas, 2, 52–59, 1897.

105. Velasco, A. L.: Geografia y estadistica de la Republica Mexicana. Mexico. 1889.

106. Venegas, J. M.: Compendio de la medicina practica en que se declara laconicum ente lo mas utile de ella que el Autor tiene observado en estas regiones de Nueva Espana, para casi todas los Enfermedades que acomben al cuerpo humano; dispuesto an forma alfabetica. Mexico. 1788.

107. Wagner, M., and C. Scherzer: Die Republik Costa Rica in Central America .... Leipzig. 1856.

108. Wells, W. V.: Explorations and Adventures in Honduras. New York. 1857.

109. Wilson, R. A.: Mexico: Its Peasants and Its Priests .... 1851–52–53–54. New York. 1856.

110. Ximinez, F.: Historia de la Provincia de San Vicente de Chiapas y Guatemala. 1666. 3 vols. Guatemala. 1929–1931.

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"OF WHOM MUCH IS REQUIRED"

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It is customary, I suppose, at the graduation exercises of a great medical school, to pay one's respects to the ancient and honorable traditions of your profession. We could start with the fabled Aesculapius, whose serpent-entwined staff is your symbol, and his daughters Hygeia and Panacea, whose names have become a part of the medical language. We might talk of Hippocrates, the first to view the doctor as a man of science, not a priest, and whose oath has become one of the most cherished traditions of your profession. Or we might talk of the great teacher, Aristotle, who a decade or so later established medicine as a part of the biological sciences—and whose most famous and hardest-drinking student was said to have been a young man later known as Alexander the Great.

We could talk of these things, but I am reasonably certain all of you are more familiar with the early traditions and the lore of your profession than I am. Let us simply settle for the statement that you are entering one of mankind's most respected professions, well prepared to take your place among the more useful and honored of our citizenry. There is every reason to expect that you will uphold, possibly enhance, the great traditions of the medical profession.

This much could in truth be said to the graduating classes of most medical colleges throughout the Nation today. What I would like to talk with you about this evening is a great tradition which is peculiarly yours—the tradition of pioneering and leadership in public affairs by the men and women of medicine in Texas. This is a tradition of which little note has been taken in recent years. Unless you revive it, there is a serious possibility that it will die out, to the great loss of Texas and your profession.

Since the great majority of the members of this graduating class are Texans and since—if you follow the example set by the more than 4,000 graduates of this institution who have preceded you—more than four out of five of you will stay in Texas to practice, I cannot, in conscience, pass up this opportunity to remind

you of your heritage and your obligation. The men of medicine played a striking role in the early development of this State and in the battle to win its freedom. You cannot evade the responsibility to carry on this role of leadership, if you are to fulfill your obligations to yourselves and your successors.

After all, the first civilized man to explore the vast wilderness which became Texas did so as a physician. When the young Spaniard, Alvar Nunez Cabeza de Vaca was washed up on the beach of Galveston Island in 1528, he had no more idea of becoming a physician than he had of exploring a Texas which he did not dream existed. However, he did both, with amazing success.

Cabeza de Vaca was pressed into service as a medicine man by the primitive Indians who inhabited this "Island of Bad Luck," as he named it (and medical students since then have been heard to claim he named it rightly). Combining an ingenious mumbo-jumbo with a strong element of faith healing, the young Spaniard enjoyed an amazing success as healer of primitive ills. This would indicate that the aboriginal Texans were either an extremely hardy lot, or that their ailments, like many today, were largely psychosomatic.

This primitive practice was lucrative. De Vaca says of his patients, in his journal, that "Their custom is, on finding themselves sick, to send for a physician, and after he has applied the cure, they give him not only all they have, but seek among their relatives more to give." As a result of this generous response, de Vaca was able to make his way from tribe to tribe, an honored and increasingly prosperous guest. His final return to the Spanish settlements of Mexico laid the foundation for Spain's claim to this portion of the New World and led to other explorations and settlements.

It was nearly 300 years before the Spanish finally got around to establishing the first hospital in Texas. I have always been intrigued, however, by the fact that when they did set up their first hospital, in 1805, they chose for its site the abandoned mission of Valero, at San Antonio—the building which we later Texans know as the Alamo.

Although this first hospital, operated by the military on a shoestring budget and dependent on supplies brought in by mule-back from the Royal Drugstore at Monterrey, survived only a

few years, it did witness another of those intriguing little events which ran through our history, in which a Texas doctor played a significant role. The hospital was still in operation in 1813 when the first of the daring filibustering expeditions headed by Anglo-Americans struck Spanish Texas. An expedition under Augustus Magee succeeded in capturing San Antonio for a few days, only to be driven out and slaughtered by troops rushed in from Mexico.

It was in the slaughter of the hapless invaders at the Medina River that a young Mexican Lieutenant named Antonio Lopez de Santa Anna learned his first lessons in no-quarter warfare. After the battle he repaired with his troops to San Antonio for a celebration, in the process of which he apparently indulged too freely in both drinking and gambling. Lieutenant Santa Anna awoke one morning to a head-splitting hangover and the freightening knowledge that he had forged his commanding general's name to a bill for his gambling debts. It was a Texas doctor, Jayme Garza, then resident in San Antonio, who bailed the lieutenant out of his trouble and probably changed the entire course of Texas history with a loan of 300 pesos.

Only six years later another filibustering expedition was being led into Texas by Dr. James Long, who formed an alliance with that noted early-day Galvestonian, Jean Lafitte, in an unsuccessful attempt to establish a republic.

When revolution did come to Texas, its doctors played a very important role. Dr. John Shackleford was one of the leaders of the defiant Texans at Goliad. Dr. Charles Ballinger Stewart was Secretary to Provisional Governor Henry Smith and later was a member of the committee which selected the design for the Texas flag and seal. Dr. James Grant was the leader of an ill-fated attempt of the Texans to invade Mexico late in 1835.

There were at least five, and possibly six, doctors among those who lost their lives at the Alamo. Of the seven doctors who signed the Texas Declaration of Independence, the most noted was Dr. Lorenzo de Zavala, Vice President of the interim government.

Fourteen Texas doctors were in active combat at San Jacinto, while two others operated a field hospital across the bayou. Most familiar of the names on this list is that of Dr. Anson Jones, Surgeon of the 2nd Regiment, who later was to serve as the last President of the Republic of Texas. Of the nine Texans who lost

their lives at San Jacinto, one was a young doctor from Kentucky, William Nottley, who was serving as an aide to General Rusk.

While Dr. Branch T. Archer held the highest military office in Texas, as Secretary of War under Lamar, probably the most noted of the "fighting doctors" of the days of the Republic, or of all time in Texas, for that matter, was Dr. John Salmon Ford. Dr. Ford came to Texas in 1836 to practice medicine, and for some months was in practice in San Augustine, but he was known to the Texans of his day and is remembered in history for his spectacular service as a fighting man. It was in this capacity that he acquired the nickname by which he is remembered and which quite possibly made it impractical for him to continue his medical career.

John Salmon Ford had served as a member of the Texas Congress and as a dashing captain of the Texas Rangers in their fights with the Indians along the frontier, when the Mexican War came along. He went into Mexico as Adjutant to Colonel Jack Hays, serving also as surgeon to the regiment. It was his duty to make out the death certificates, and feeling that some formality was appropriate to such a serious document, Ford concluded each with the phrase, "Rest In Peace." Soon the fighting became fierce and casualties mounted. Ford had too many death certificates to fill out and too little time for the chore. He shortened his conclusion to each certificate to "R. I. P., John S. Ford."

The soldiers soon began calling him "Rip," and the nickname stuck for the rest of his life—a suitable title for a fighting man, but a poor recommendation for a doctor.

These early Texas doctors were a rugged lot. As a whole, they were poorly trained, by today's standards. Most of them came up through the old preceptor system, spending a few years in the office of an established doctor and possibly taking a year or two at one of the many small pivately-operated medical schools which flourished in this period. They were notorious for their dependence on calomel and quinine, given in heroic doses, and made free use of native remedies which they picked up from the Indians. Quackery was rampant. Dr. Theodore Leger, in the first known medical publication in Texas, an 18 page pamphlet published in 1835, refers to "the innumerable horde of Prophets,

Sophists, Hypocrites, Pedants, Pettifoggers, Politicasters, Charlatans and Quacks who infest this Republic."

Still there was a sprinkling of extremely well-educated and scholarly men among them. Most notable was Dr. Ashbel Smith, a graduate of Yale who had done post-graduate work in Paris before coming to the new Republic in 1837. Dr. Smith left his mark on the history of Texas as a public figure, medical scholar, and leader in the establishment of public education. He served as Surgeon General of the Texas Army, soon after coming to Texas. As a diplomatic representative of the Republic in England, France, and Mexico he is credited with laying the groundwork for the eventual annexation of Texas to the United States. His "Account of the Yellow Fever Which Appeared in the City of Galveston, Republic of Texas, in the Autumn of 1839, With Cases and Dissections," is recognized as the first great medical book published in Texas.

Possibly his greatest service to Texas was in the fostering of medical education and of higher education in general. He served as a director of the Texas Medical College at Galveston, which was later to become the Medical Branch of the University of Texas. The chief labor of his later years was for the establishment of the University of Texas, for which he furnished inspiring leadership as President of its Board of Regents.

The Texas of today owes a great debt to its pioneer leaders from the ranks of the medical profession. Can it look to you to carry on this proud tradition?

There has been a tendency in recent years for the members of the medical profession to hold aloof from public life. The word "Politics" has become an epithet, besmirched by the general acceptance of the inevitability of corruption in public office. Doctors, nurses, and others of the medical profession have withdrawn more and more from political activity, as though fearful of being contaminated by the unclean.

I would like to remind you that political activity is simply the process by which we govern ourselves—or allow ourselves to be governed. And let there be no mistake about this; the minute we fail to govern ourselves, there will always be someone available to take over the task for us.

There is no power inherent in government, just as there is no wealth produced by government. Government secures both

power and wealth originally as gifts from the people. Yet, by its very nature, government is dedicated to the acquisition of even more power and wealth, both of which it will take from the people to the extent which they will allow. Unless there is a constant vigilance on the part of the people to exercise their rightful powers and control their own wealth, government inevitably gobbles up both until its power is absolute and it controls all wealth. When this extreme is reached the people are powerless, and poverty-stricken, the slaves of a system of their own creation. Slaves create little wealth; government none. Such a system inevitably falls.

Our American form of government was established to enable us to govern ourselves. Our Constitution was drawn up by men who hated and feared the evils of a strongly centralized, power-grasping government. They placed in the original document more safeguards against the development of such a government than had ever before existed in a constitutional document.

The whole system was built, however, upon the theory that we, as people, would continue to cherish and safeguard our individual freedoms and defend those freedoms against any encroachment from within or without. This system required the continuing participation of our citizenship in the political process of establishing governmental policy. It assumed that Americans would exercise and defend their hard-won rights of free citizenship.

But what has happened? We, as citizens, have ignored the responsibilities of politics. We have been too busy, too lazy, too indifferent, to exercise our powers and protect our prerogatives. Into the vacuum created by our failure to exercise our power has naturally seeped the bureaucracy, fulfilling the functions which should be fulfilled by the citizenry and must be fulfilled by someone. Bit by bit, through amendment and interpretation, our sturdy constitutional safeguards have been eroded.

Small wonder that we have drifted into a system in which the average citizen often feels lost and helpless. Small wonder that we have crept inch by inch toward the socialization of many fields, including medicine. Small wonder that the once respected and vitally important concept of "states rights" has become outmoded.

And who is to blame? We are to blame—you and I and the

millions of other Americans who have become too busy to attend to the important business of governing ourselves—too lazy to make the effort of studying, discussing, and voting on the issues which determine our governmental politics—too indifferent to defend our individual freedoms against the malignant growth of Big Government.

We are trodding the old primrose path of many nations of history, toward more and more government, less and less individual freedom and responsibility. This is the really great threat which we face today—far greater than any threat posed by the Communists. If we meet this threat—if we prove to the world that our form of government can and will work as it was designed to work, we need have no fear of Communism or any other outside force. If we dodge this issue, shrug off our responsibilities, seek only our own immediate selfish ends, then we will fall of our own weight, just as the Communists have said we would from the beginning.

This is the area in which the battle for freedom will be fought in the future. I feel it is only proper for me to warn you that Texas will look to your profession to supply a fair share of the leadership in this battle, just as it has in the battles of the past.

I think you who have received your professional education at a tax-supported institution have an added obligation to your State and Nation. In the twelfth chapter of Luke we read the story of the faithful servant, and at the close, these words: "Everyone to whom much is given, of him much will be required." This is the law of proportionate responsibility.

Each of us has more of something than another: more time, more money, more education, more ability, or more talents—of us, more is required. If your opportunities have been greater, your experience more varied, if your horizon is thereby broadened, your preception keener, your vision and insight more clear, then your responsibility and obligation to give of the fruits of your life is greater. Great gifts should make one humble and aware of his obligation to give more.

I feel sure you are prepared and willing to assume this responsibility, and may God bless each of you as you walk in the pathway of the Great Physician.

* Commencement Address, Medical Branch, The University of Texas, Galveston, Texas, May 28, 1959, M. T. Harrington, President, Texas A. and M. College System.

THE ROLE OF THE PRIVATE PHYSICAN IN VENEREAL DISEASE CONTROL

C. HUNTER MONTGOMERY[a], WILLIAM O. HOSKING[b], AND FRED K. LAURENTZ[c]

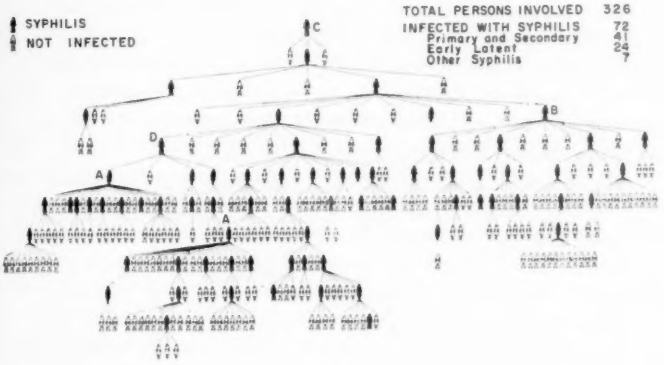
With the dramatic decline in the incidence of syphilis following the advent of penicillin, there was a relaxation of syphilis control programs throughout the country. Budgets were slashed, personnel reduced, public clinics closed and the carefully developed epidemiological apparatus that brought the patient and the penicillin together was undermined or scuttled. The syphilis rate was declining so rapidly that it seemed that syphilis would melt away even if all programing ceased. Anybody could get and take penicillin and it seemed that everyone was doing just that.

The ease of treatment not only brought the actual incidence of syphilis down but also created an artificial decrease in the reported cases. Whereas previously the majority of patients suffering from syphilis were diagnosed and treated at public clinics where they were reported, the syphilis patient began to migrate to the private practitioner, not only in areas where the public clinic had been closed but where they remained open as well. This presented no problem in treatment but cases were not reported and as this migration continued and programs were restricted, the statistics came tumbling down. Syphilis, the great scourge of humanity, was on its way to oblivion.

Then in 1956 the United States Public Health Service reported the first increase in syphilis since 1947. This was followed by another increase in 1957. What had gone wrong? No one seriously believed that syphilis would be eradicated from the population but the general consensus seemed to have been that the disease would quickly decline to a point where it would cease to be a public health problem as have so many communicable deseases in the past. Apparently forgotten was that which makes the venereal disease infinitely more difficult to control than other contagious disease—the mode of transmission.

While it is possible to contact syphilis without indulging in promiscuous sexual activity, it is well known that such activity is

the primary factor in the spread of the disease. As long as there is sexual promiscuity and a reservoir of infectious syphilis in a community, outbreaks of infection are bound to occur. The accompanying chart depicts a chain of infection of syphilis graphically illustrating this point. Of the seventy-two infected persons in this chain of infection only nine have no contacts other than the person who originally named them. Five of these nine were noninfectious and hence not interviewed for sex contacts. Of the remaining four persons, one was treated by a private physician and not available for interview. The remaining three persons de-



nied any contact other than the one who had originally named them. These three, however, are in sharp contrast to what appears to be the normal pattern among people involved in the chain of the infection. The contact index (average number of contacts named per patient interviewed) on all patients with primary and secondary syphilis was 6.39 with the females contributing 7.87 and the males 5.44. The median and the mode were also significantly higher for the females interviewed than the males. Both the median and the mode were 7 for the females and 4 for the males. In cases of primary syphilis, interview extended back 3 months; for secondary syphilis, back 6 months, indicating the frequency of a new sexual partner to be about once a month. We have no idea of the frequency per partner.

Most of the females in the chain defy classification in the usual dichotomous division into those who are simply promiscuous and

those who are frank prostitutes. In general these females were not prostitutes in the usual sense of the word. None of them worked out of a house of prostitution and most had full time jobs unassociated with sexual activity. In most instances they set no actual price for their services but most expected to "go out on the town" and many let it be known that some gratuity for the services rendered would be welcome. There were only two notable exceptions. The two females designated (A) on the chart used sexual activity as their primary means of livelihood and the taverns as their market place. They complicate the problem of syphilis control. Even though they were able to furnish information about many contacts, 20 and 21 respectively, both admitted that undoubtedly there were others whom they could not remember.

While the contact indices in this chain of infection would indicate that the females in this group were more promiscuous than the males, it may actually be that the females are more amenable to interviewing techniques. The females contribute a special problem in the control of syphilis with the prostitute who could not remember her sex partners; the males with the homosexual, who because of the social, moral, psychological and legal implications do not wish to divulge that they engage in this type of activity much less name their sexual partners. The difficulty is compounded by the fact that, despite the generally accepted opinion to the contrary, it is usually not possible to tell a homosexual by his appearance. Yet this chart illustrates homosexual activity to be a contributing factor in the spread of syphilis (B).

The problem of the control of syphilis revolves around two factors (1) sexual promiscuity and (2) a reservoir of infectious syphilis. There is no indication that sexual behavior is changing and the chances of instilling some degree of sexual responsibility or restraint on the populace is difficult, if not impossible. If we are to get syphilis under control and keep it there, we must somehow reduce the reservoir of infectious syphilis and remain constantly on guard to prevent infections that do occur from becoming epidemic in proportions.

That reservoirs of infection remain is evidenced by the continued occurrence of primary and secondary syphilis. The size of the reservoir is unknown but would appear to be large. During 1956 only five cases of syphilis were reported from one county in Texas, yet the state laboratory servicing the county reported

503 definitely reactive and 351 doubtful serologic tests for syphilis. A check of private facilities in the area would have made the total even higher.[d] If only 3% (15 cases) of the 503 definitely reactive reports from the state laboratory represented infectious syphilis, the infectious reservoir would have been twice the infectious and noninfectious syphilis reported. On the other hand, the size of the reservoir is exemplified by the dramatic increases that occur when aggressive case finding programs are instituted. Within 2 years after intensification of the venereal disease program in Houston, Texas, reported cases of primary and secondary syphilis increased over 400% (40 to 175 cases per year).

Unfortunately reservoirs of infection are not static but continuously perpetuated from individual to individual as demonstrated in the preceding diagram. Any reduction of such a reservoir is minimal if the individual patient is treated as a distinct entity. Epidemiologic investigation of all infectious cases is vital. Fundamental to venereal disease control, however, is the discovery of the infectious case to serve as a point of entry into these dynamic, continuously enlarging reservoirs.

When syphilis was rampant and treatment became simplified, control programs could and did achieve dramatic reductions in the incidence of syphilis with little reference to the relatively small percentage of patients treated by the private practitioner. With the use of mass blood testing surveys, cases were discovered, diagnosed, treated and epidemiologic investigation made with little in the way of assistance from the private physician. Today, with the reduced incidence of the disease, mass surveys have, except in localized geographic areas or special groups, reached a point of diminishing returns.

In the past a great deal of emphasis has been place upon required blood testing such as pre-marital, pre-natal and pre-employment examinations and hospital admissions. These testing requirements have been valuable and remain so provided discovered cases are reported to health departments in order that epidemiologic investigations may be carried out. This fact is well demonstrated by the chain of infection cited. The male designated (C) from whom our entire sequence of infection began was reported to us as a result of a reactive serology on his preinduction physical examination. All too often these tests have been used for diagnosis and treatment of only the individual patient. Private

practitioners have not yet been acquainted with concepts of epidemiology sufficiently to realize the necessity for reporting venereal disease. Failure to report such cases only allows for continuous spread. This is demonstrated by the male labeled (D) in the accompanying chart. This man was treated by his family physician who made no epidemiologic investigation nor did he report the case. Two months later he was brought to our attention by one of his contacts. Had this case been reported to us at the time of treatment, this contact and many others might have been detected and treated sooner, thereby reducing the spread of infection.

With the bulk of our cases removed from the reach of our previous control techniques, we must shift our attention to a wider field of endeavor, the private physician, as a source of epidemiologic material. Our previous techniques, while of immense value in the initial phases of control, appear no longer adequate for the final phase—eradication. We must call upon the vast and broad resources of our colleagues in therapeutic medicine.

In the past when diseases have declined in incidence or disappeared, it has usually been the custom to view with alarm the occurrence of even a single case. The appearance of only a few cases of smallpox, typhoid or yellow fever would be considered an epidemic. Yet many cases of infectious syphilis occur annually with little attention directed to their source or subsequent spread. Cannot more concern be directed to early syphilis? We not only feel that it can but must be if we are to finish the task of eradication. It is our job in public health to impress upon our colleagues in practice the communicable nature of the disease and outline a cooperative program of control. In those instances in which we have explained our need, private practitioners have been more than willing to provide assistance. Such individual participation is not enough. We must endeavor to set up programs on a wider scale through local and state medical societies.

It is too much to expect the physician in practice to handle the medical aspects of patient care and in addition perform an epidemiologic investigation with which he is unfamiliar. It is not unreasonable to expect that he report cases treated and request epidemiologic help. It is these reports which will provide our entry into our reduced but still building reservoirs. Most, if not all, states have laws demanding such reporting; but laws are not

the answer. The answer is to be found in reorientation of venereal disease control programs toward mutual cooperation with the private practitioner.

Since we cannot control the mode of transmission for syphilis, elimination of our infectious reservoirs is our only recourse to eradication or effective control. As these reservoirs become smaller, they become more difficult to find. As syphilis patients continue this migration from public clinics to the private physician, we are losing our keys of entry into these even more difficult to locate sources of disease. We in public health must prepare ourselves for private physician orientated programs since only by discovery of the remaining reservoirs of disease can they be eliminated.

Acknowledgment

We are indebted to Dr. Allen R. Doane, former Medical Director, Division of Venereal Disease Control, Texas State Department of Health, upon whose knowledge and views we relied extensively in the preparation of this paper.

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[d] Doane, Allan R.: "The Status of Venereal Disease Control in Texas," *Texas Reports on Biology and Medicine*, Volume 15, Number 4, page 842, Winter, 1957.

THE GASTROINTESTINAL TRACT AND MUSCULAR DYSTROPHY

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The gastrointestinal tract is rarely mentioned in commentaries on progressive muscular dystrophy. Our interest in this subject was stimulated by the care of a young woman with what appeared to be progressive muscular dystrophy and an associated disturbance of gastrointestinal motility. This case has been reported in detail elsewhere (24). In searching the medical literature for help in the management of this particular patient, we discovered enough references to the gastrointestinal tract and muscular dystrophy to encourage us in a systematic survey for such reports and to review our own experience. This article is a summary of our findings.

Muscular Dystrophy and Gastrointestinal Symptoms. One of the earliest reports of a patient with muscular dystrophy and associated gastrointestinal symptoms is that recorded by Ross (1883). This patient was a ten year old boy with pseudohypertrophic muscular dystrophy who developed a severe attack of diarrhea and vomiting accompanied by great prostration. Death followed three days later. At post-mortem examination, the large intestine and the lower half of the small intestine were distended with hard fecal matter. No microscopic description of the intestine is recorded.

Cardarelli (1896) reported the cases of a 15 year old dystrophic male who had severe attacks of diarrhea and prostration which led to a sudden decline and death. At autopsy, there was ulceration of the large intestine.

Globus (1923) reported a case of a five year old male with progressive muscular dystrophy who after a tooth extraction collapsed with a rapid pulse. There was continual vomiting, followed by a convulsion and death twenty-four hours later. At post mortem the stomach and small intestine were normal. The large intestine was distended by solidified feces. No microscopic examination of the intestine is recorded.

Hurwitz (1936) reviewed 36 cases of primary myopathy.

Death from gastroenteritis was observed in one patient. Another patient had troublesome constipation and another dysphagia. No details of the illness are given.

Bevans (1945) noted fecal impaction and hemorrhage of the rectal mucosa in one patient. She reported another patient who had recurrent attacks of abdominal pain, vomiting, diarrhea, and fecal impactions for three years prior to death. No autopsy was permitted.

Gornia and DeGispert (1948) stated that on various occasions they had noted atony and atrophy of the esophagus and meteorism in patients with dystrophia myotonia. Frequent diarrhea was a problem in one case.

Bertrand (1949) reported five instances of megacolon diagnosed by barium enema examination in dystrophia myotonica patients. One of these patients had an associated megaesophagus. The details of the symptoms are not given but one patient did develop a volvulus.

We have reviewed the medical records of 46 patients with progressive muscular dystrophy seen at this university. Thirty-one of these patients were diagnosed as pseudohypertrophic muscular dystrophy and three were patients with dystrophia myotonica. There were 33 males and 13 females, and 9 were Negroes. The majority were children (70%). The mean age was 15 with a range of age from 3 to 58 years, and 70% of the group were children.

Complaints referable to the gastrointestinal tract were recorded in 5 patients but only 1 patient was investigated with gastrointestinal roentgen ray examination. Dysphagia of a mild degree was noted in two instances. Both of these individuals were adults, one was a case of myotonica.

Constipation was troublesome in a five year old boy with pseudophyertrophic muscular dystrophy. A fourth patient, previously referred to in the introduction (24), was a twenty-six year old female thought to have progressive muscular dystrophy of the juvenile type of Erb who had severe vomiting. Chronic duodenal stasis was demonstrated by roentgen ray and was interpreted as a result of superior mesenteric artery compression. Following duodenojejunostomy, gastric retention and delayed motility continued for several months. Later, death occurred as a result of inanition. An autopsy, done elsewhere, showed the

musculature of the gastrointestinal tract to be involved in the same process that involved the skeletal muscle. The final diagnosis of progressive muscular dystrophy or polymyositis could not be made with certainty.

The fifth patient, and the only patient with progressive muscular dystrophy that had died and had a post-mortem examination in this hospital, had a very similar illness to a few cases reported by others (4, 11, 10, 25).

This 15 year old orphan boy was under domiciliary care in our hospital from the 22nd of July, 1926, to the time of his death on January 7, 1929. He was the youngest and only son of a family of six. His mother died when he was one month old and his father died the year before his hospital admission. The history was taken from the child and does not include information regarding his lineage or early childhood development. At the age of nine, he had noticed generalized weakness, slowness in getting about, and easy fatigue. These complaints had progressed to the point that he could walk only a short distance when his legs would collapse beneath him. On admission, he was unable to care for himself. The physical examination revealed a typical picture of far advanced pseudohypertrophic muscular dystrophy with considerable atrophy of the upper extremities, protuberant abdomen, lordosis, broad stance, and hypertrophied calf muscles. There were some flexure deformities of the legs. During his hospital stay, there were no important symptoms recorded except that he became progressively weaker.

On December 28, 1928, his temperature and pulse rose and he began to complain of severe lower abdominal pain. There was associated abdominal distension and a suggestion of muscular rigidity, particularly in the right lower quadrant. The white count was 9,600 with 65% neutrophiles. A roentgen ray examination of the chest on the 31st of December revealed a heart "greatly enlarged" and a marked increase in perivascular and peribronchial markings. The physician who saw this patient made a diagnosis of acute appendicitis. A laparotomy was done on December 31, 1928, with negative findings. A normal appendix was removed. After surgery, his condition deteriorated. His lower abdominal pain persisted, abdominal distension became worse, his temperature elevation and marked tachycardia persisted and near the end of his illness he vomited blood. On

January 4, there was evidence of bilateral bronchopneumonia by physical examination and the chest rotentgen ray. On January 7, 1928, he died. The autopsy diagnosis was bilateral bronchopneumonia, purulent pyelitis, fatty degeneration of the liver, cloudy swelling of the kidney, and heart, and atrophy and fibrosis of the voluntary muscles. No abnormalities of the gastrointestinal tract were noted and no cause was found for the hematemesis. These tissues are not available for review.

It may be argued that the gastrointestinal symptoms were a reflection of referred pain and ileus from bronchopneumonia, yet at the onset of the abdominal symptoms the chest was reported to be normal by physical examination, and the roentgen ray revealed no evidence of such a process.

A similar case has been reported by Berblinger and Duken (1929). Their patient was a fourteen year old boy who developed sudden vomiting, generalized abdominal tenderness, and an increasing pulse and temperature. Because of constipation for 24 hours, enemas were given with good results but without symptomatic improvement. The pulse rate rose to 140/minute and the vomiting increased. There was abdominal tenderness with localization to the right lower quadrant. In the middle of the night, an emergency laparotomy was done with negative findings. The following morning the pulse was small and irregular. The general condition of the patient was poor. Hematemesis occurred. The following afternoon, the patient died. At autopsy, aside from changes noted in the heart muscle, there was erosion of the gastric mucosae with bloody stomach contents and atony. No microscopic findings of the gastrointestinal tract are recorded.

This boy and his brother gave a past history of episodes of pallor, tachycardia, and vomiting. Electrocardiographic studies revealed episodes of paroxysmal ventricular tachycardia. These authors explained the gastrointestinal symptoms on the basis of cardiovascular disease although the symptoms had their onset before the tachycardia of any marked degree was noted.

A third child, also reported by these writers (4), had severe constipation regulated by means of enemas with occasional periods of fecal incontinence, labile temperature changes, and episodes of tachycardia.

Constipation and fecal impaction developing in bed-ridden patients with poor abdominal muscle tone seems readily under-

standable, but that this condition should progress to such a degree that death ensues is unusual and worthwhile emphasizing. The other gastrointestinal symptoms noted are difficult to explain on the basis of information available regarding the muscular dystrophies.

Muscular Dystrophy and the Gastrointestinal Musculature. The muscles of the tongue and the upper esophagus may be involved (6, 26, 32) in muscular dystrophy, but it is generally stated that smooth muscle is not affected by the changes observed in the striated and cardiac muscles (1, 26). Yet changes have been reported.

Bunting (1908) observed changes in the muscular wall of the stomach which he felt were "quite similar in character to those in the striated muscle, although much less extensive." These changes consisted of numerical atrophy of fibers, invasion of fasciculi by connective tissue, while other fibers were vacuolated and some appeared hypertrophied.

Bevans (1945) reported four patients in which autopsy disclosed abnormalities in the gastrointestinal musculature. In two of these patients, the lower end of the esophagus had marked edema of all layers with muscle fibers that were indistinct in outline and variable in size and staining quality. These fibers were often disarranged. The cytoplasm appeared vacuolated and nuclei were stretched into wavy filaments. In two patients, the stomach was greatly dilated and muscle changes occurred that were similar to those described in the esophagus. The small intestinal changes were atrophy with thinning of the wall, edema and disappearance of muscle fibers. These changes were less severe than in other parts of the gastrointestinal tract, while in the rectum, they were most marked. The plexus of Auerbach and Meisner appeared normal.

Bevans commented that these changes might be a reflection of nutritional disturbance or of severe debilitation and that they were in no way pathognomoic of progressive muscular dystrophy. Nevertheless, she suggested that they might explain the symptoms that have been noted in some of these patients.

Lewitan and Nathanson (1945), reporting on the roentgen features of muscular dystrophy stated that to a limited extent, replacement of the smooth muscle within the intestinal wall had been seen, but there were no consistent findings on roentgen ray

examination to demonstrate this involvement. Occasionally, they had noted diminished peristalsis in the esophagus.

When one considers the extent of the smooth muscle in the gastrointestinal tract and the rapid deterioration of the morphology in this organ after death, it does not seem inconceivable that changes could occur that have been overlooked. We have not found any reports of a systematic study of gastrointestinal histology in these patients other than Bevans. Until other such studies are done, it would perhaps be wiser to withhold a final opinion about the gastrointestinal musculature and muscular dystrophy.

Muscular Dystrophy and Gastric Juice. Goettsch and Pappenheimer (1931) produced a myopathy histologically similar to muscular dystrophy in animals fed Vitamin E deficient diets. This stimulated further workers to investigate the importance of this substance in human muscular dystrophy. Disappointingly, Vitamin E did not affect the clinical symptoms (23) or the chemical abnormalities (20.21) of this disease. Nor is there any evidence to indicate that a Vitamin E absorptive defect exists in these patients (23).

Milhorat and Bartels (1945) have shown that when alpha-tocopherol is ingested by normal subjects and removed thirty minutes later and then administered to dystrophic patients a decrease in creatinuria sometimes occurred. A decrease was also noted for periods from eleven to sixteen days when fresh concentrated hog gastric mucin and alpha-tocopherol were fed to muscular dystrophy patients. A similar biologically active substance was isolated from the hog stomach lining (22). These findings and other studies (4) were interpreted as indicating a deficiency of some gastric substance necessary for the reaction of condensation of the tocopherol. This deficiency thus might prevent the utilization of tocopherol by muscle.

Determination of group-specific substances in gastric mucin by methods based on techniques used for blood grouping revealed no significant differences in the mucin of muscular dystrophy patients and normal individuals (22). However, Wolf and others (1956), using the principle of ion-exchange chromatography have reported differences in the gastric mucin of three patients with dystrophia myotonica. This difference consisted of an absent protein peak at pH 6.1. The significance of this difference has not been fully explored.

General discussion

Muscular dystrophy remains a medical enigma. There are three theories proposed to explain its pathogenesis (28):

1. It is a heredito-familial disease—perhaps an intrinsic genetic defect of striated muscle
2. It is a poorly defined endocrine disturbance
3. It is the result of an imbalance of sympathetic and parasympathetic nerve activity

The hereditary basis for muscular dystrophy appears incontrovertible, but whether this defect is primarily muscular or whether the changes observed in muscles are secondary to a metabolic defect or a specific deficiency is not established.

The involvement of different groups of muscles in different types of dystrophy is strong argument for a specific genetic defect. However, the work of Leonard and Wortman (17, 18, 31) suggests that the concept of an identical metabolic response in all striated muscle may not be valid. For example, it has been observed in rats that different groups of muscles vary in their glycogen content in response to steroid hormones, castration, or hypophysectomy. One might speculate that a specific deficit could result in variable changes in musculature, with some muscle groups being more sensitive to this deficit than others. This might explain the lack of cardiac changes in all cases of muscular dystrophy and the rarer changes in the gastrointestinal muscle. Certainly, death would ensue very early if the heart muscle was involved as extensively as skeletal muscle in dystrophic patients. The patient could not survive myocardial damage to permit that degree of change. A search for a nutritional or metabolic deficit has more optimistic therapeutic implications than defective somatogenesis.

Many neuromuscular disorders which resemble muscular dystrophy have been described in association with various endocrine disorders (2, 3, 7, 8, 15, 27), but Tyler and Perkoff (1951) were unable to demonstrate any abnormality of carbohydrate metabolism, adrenal cortical function, thyroid or gonadal function in a series of muscular dystrophy patients they investigated. They conclude that there is no evidence in their experience to indicate that this disease is a primary endocrine disorder.

The concept of autonomic nervous system imbalance or defect

is an attractive explanation of the labile cardiovascular symptoms that have been noted in these patients. It would also explain the bizarre gastrointestinal manifestations. As far as we have been able to determine, only Kure (1928) has presented any evidence toward this concept.

Conclusion

The literature and our own experience in regard to muscular dystrophy and the gastrointestinal tract has been summarized. We are impressed by the lack of statements regarding this subject in most standard works on this disease in spite of what we consider to be some rather dramatic case reports in which gastrointestinal symptoms predominated. We are unable to accept a dogmatic statement that the smooth muscle cannot be involved in this disease on the basis of the studies that we have read. Particularly, when one considers the extent of this musculature and the usual number of sections that are examined in routine postmortem examinations of the gastrointestinal tract. We cannot say whether the findings noted in the reports reviewed are primary, secondary or fortuitous, but further and more detailed studies seem warranted. Observations relating to gastric juices and muscular dystrophy have also been reviewed.

REFERENCES AND NOTES

1. Adams, R. D., D. Denny-Brown, and C. M. Pearson: Muscular Dystrophies in Diseases of Muscle, New York, Paul B. Hoeber, Inc., 1954.
2. Barris, R. W.: Hyperinsulinism and Nueromuscular Disorders—A Consideration of the Association of Pancreatic Adenoma with Wasting States. Calif. Med. 78:224, 1953.
3. *IBID.* Pancreatic Adenoma (Hyperinsulinism) Associated with Neuromuscular Disorders. Ann. Int. Med. 38:124, 1953.
4. Berblinger, Prof. Dr. and Prof. Dr. Duken, Derkàrdio-intestinale symptomenkomplex bei der progressiven Muskeldystrophie; 1. Mitteilung. klinische und pathologischanatomische Beobachtungen. Ztchr. fur Kinderh. 47:1, 1929.
5. Bertrand, L.: Le megacolon dans la maladie de Steinert. Rev. Neurol. 81:480, 1949.
6. Bevans, Margaret: Changes in the Musculature of the Gastrointestinal Tract and in the Myocardium in Progressive Muscular Dystrophy. Arch. Path. 40:225, 1945.
7. Bramwell, E.: The Muscular Dystrophies, Sympathetic System and Endocrine Glands. Lancet 2:1103, 1925.
8. Brock, S. and W. E. Kay: Study of Unusual Endocrine Disturbances—Their Associated Myopathies, Endocrine Balance and Metabolism Findings, Arch. Int. Med. 27:1, 1921.

9. Bunting, C. H.: Chronic Fibrous Myocarditis in Progressive Muscular Dystrophy, Am. J. M. Sc. 135:244, 1908.

10. Cardarelli, N.: Contributo allo studio clinico ed anatomo-pathologica delle distrofi e muscolari progressive Policlin. Roman III-m:453, 1896 (cited by Globus).

11. Globus, J. H.: The Pathological Findings in the Heart Muscle in Progressive Muscular Dystrophy. Arch. Neurol. & Psychiat. 9:59, 1923.

12. Goettsch, Marianne and A. M. Pappenheimer: Nutritional Muscular Dystrophy in the Guiena Pig and Rabbit. J. Exper. Med. 54:145, 1931.

13. Gorina, A. B. and Gurz DeGispert: La Distrofia Miotonica Como Affection General y en sus Aspectos Otoneuro-oftalmologicos. Rev. Espanola de Oto-Neuro-Oftl. y Neurocir. 7:7, 1948.

14. Hurwitz, S.: Primary Myopathies Report of 36 Cases and Rev. of Literture. Arch. Neurol. & Psychiat. 36:1294, 1936.

15. Janney, N. N., Goodhard, S. P., and V. I. Isaacson: The Endocrine Origin of Muscular Dystrophy. Arch. Int. Med. 21:188, 1918.

16. Kure, Ken: On the Pathogenesis of Dystrophia Musculorum Progressiva. Lancet, 1:441, 1928.

17. Leonard, S. L.: A Glycostatic Effect of Testosterone of the Perineal Muscles of the Rat. Endocrinology, 50:199, 1952.

18. *IBID.* Glycogen Deposition in Different Skeletal Muscles Induced by Cortisone and Estradiol. Endocrinology, 53:226, 1953.

19. Lewitan, A. and L. Nathanson: The Roentgen Findings of Muscular Dystrophy. Am. J. Roentgenol., 73: 226, 1955.

20. Milhorat, A. T., V. Toscani, and W. E. Bartels: Effect of Wheat Germ on Creatinuria in Dermatomyositis and Progressive Muscular Dystrophy Proc. Soc. Exper. Biol. & Med., 58:40, 1945.

21. Milhorat, A. T. and W. E. Bartels: The Defect of Utilization of Tocopherol in Progressive Muscular Dystrophy. Science, 101:93, 1945.

22. Milhorat, A. T., J. B. Mackenzie, S. Blick, H. Rosenkrantz, and W. E. Bartels: Observations on a Biologically Active Vit. E Derivative Present in Hog Gastric Mucin and in Hog Stomach Lining. The Biological Activity of DL. Alpha-Tocopherylhydroquinone. Ann. New York Acad. Sc., 52:334, 1949.

23. Minot, A. S. and H. E. Frank: Serum Tocopherol—Its Relation to Failure of Vitamin E Therapy for Pseudohypertrophic Muscular Dystrophy. Am. J. Dis. Child., 67:371, 1944.

24. Patterson, Marcel and Gustavo Rios: Disturbed Gastrointestinal Motility —An Unusual Manifestation of a Systemic Muscular Disorder Polymositis or Progressive Muscular Dystrophy, Report of a Case. Gastroenterology, 36:2, February, 1959.

25. Ross, James: On a Case of Pseudophyertrophic Paralysis. Brit. M. J. 1:200, 1883.

26. Shank, R. E., H. Gilder, and C. L. Hoagland: Studies on Diseases of Muscle I. Progressive Muscular Dystrophy. A Clinical Review of Forty Cases. Arch. Neurol. & Psychiat., 52:431, 1944.

27. Timme, W.: Progressive Muscular Dystrophy as an Endocrine Disorder. Arch. Int. Med., 19:79, 1917.

28. Tyler, F. H. and M. M. Wintrobe: Studies in Dosorders of Muscle I. The Problem of Progressive Muscular Dystrophy. Ann. Int. Med., 32:72, 1950.

29. Tyler, F. H. and G. T. Perkoff: Studies in Disorders of Muscle IV. Is Progressive Muscular Dystrophy an Endocrine or Metabolic Disorder. AMA Arch. Int. Med., 88: 175, 1951.

30. Wolf, S. R., R. Caputto, V. Richmond, and L. Russell: The Fractionation of Important Constituents of Gastric Juice Other than Hydrocholoric Acid. Ann. Int. Med., 45:825, 1956.

31. Wortman, L. C. and S. L. Leonard: The Effect of Injections of Testosterone Proprionate and Cortisone Acetate on Glycogen Depletion Induced by Epinephrine in Different Skeletal Muscles. Endocrinology, 53:480, 1953.

32. Yoshida, Tomizo: Uber die Myokardveranderung bei der Progressiven Muskeldystrophie Jap. J. M. Sc. V. Path., 5:63, 1940.

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EFFECTS OF HYPOTHALAMIC LESIONS ON ENDOCRINE ACTIVITY IN FEMALE RATS[a]

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Introduction

The role played by the hypothalamus in the regulation of endocrine function has received much attention, especially with regard to the mechanisms and the areas of the hypothalamus involved in the control of the anterior pituitary. The following experiments were done to gain further insight into the specific role of the hypothalamus in endocrine function, especially in regard to reproductive function.

Various methods have been used to study the relationship between the hypothalamus and the hypophysis. Pituitary stalk section and pituitary transplantation have been used to study this relationship, especially by Harris and his coworkers (1955). Also, in recent years, the nature of this relationship has been investigated by electrical and pharmacological stimulation of the hypothalamus and by lesions placed in the hypothalamus. Lesions in the hypothalamus, although presenting more problems in interpretion of results, produce more consistent results with fewer technical problems. It is this method of hypothalamic lesions that has been used in the present study.

As a result of the earlier inconclusive work of Bailey and Bremer (1921), Dey and his coworkers (1940) did the first systematic study of the effects of hypothalamic lesions on reproductive function. In a large group of female guinea pigs they found that large lesions of the anterior hypothalamus would produce a state of constant estrus. Dey concluded that the large anterior lesions interfered with the secretion of luteinizing hormone (LH) and that continuous release of follicle stimulating hormone (FSH) plus small amounts of LH would produce a constant estrous state. They also described lesions of the median eminence which produced acyclic animals with genital atrophy and suggested that this resulted from interruption of connections between the hypothalamus and the hypophysis. About one half

of Dey's animals showed regular estrous cycles, although many would not mate or become pregnant.

Little work was done on this problem during the following years until 1949 when Hillarp reported similar findings in the rat. Hillarp used smaller, more discrete lesions and was thus better able to localize specific areas which when destroyed would produce these altered estrous cycles. He found that large anterior hypothalamic lesions between the paraventricular nucleus and the anterior hypothalamic area would produce the constant estrous state, but also that very small lesions between the paraventricular nucleus and the hypophysial stalk would also produce this change. He concluded that the smaller lesion interrupted a fairly well demarcated fiber system running on both sides of the median eminence towards the hypophysial stalk, and the larger lesion destroyed "a center." The ovaries of these animals contained estrogen producing follicles, no corpora lutea and extremely well developed interstitial tissue. On this basis he concluded, as had Dey, that the constant estrous state was produced by a reduced LH secretion. Eleven of Hillarp's animals showed cycles characterized by long periods of diestrus (persistent diestrus). These animals had lesions generally located anterior to the paraventricular nucleus. These animals did not become pregnant, but the ovaries showed no demonstrable changes, both follicles and corpora lutea being present. In all of his animals, Hillarp reported no thyroid, adrenal or gonadal atrophy.

Soon after Hillarp's work several workers published on hypothalamic lesions and the effects on the thyroid and adrenal gland (Bogdanove and Halmi, 1953, and Greer, 1956). Separate thyrotrophin (TSH) and adrenocorticotrophin (ACTH) centers have been fairly well localized, and although lesions of these centers will not produce glandular atrophy, compensatory hypertrophy and physiological responses have been abolished.

Bogdanove (1955) noted testicular atrophy in 26 of 59 lesioned male rats. The exact location of these lesions was not reported, but four of these animals had severe testicular atrophy as well as atrophy of the adrenals and thyroids, to hypophysectomy levels. On the basis of total gonadotrophin assays, Bogdanove concluded that all lesioned animals with marked testicular atrophy had no demonstrable gonadotrophin. He also reported that gonadotrophs,

although present in the pituitaries of animals with marked atrophy, were very sparse.

This gonadal atrophy, seen by Dey in the female guinea pig and Bogdanove in the male rat, has not been seen by other workers in female rats, and Greer (1958) concluded that this was due to a sex difference in the relative dependence of gonadotrophin secretion upon the hypothalamus. Greer also reported that animals in constant estrus produced by lesions would, like those animals of Everett (1943) in spontaneous constant estrus, resume normal cyclic activity after daily 0.5–1.0 mg. injections of progesterone, and that this normal cyclic activity would continue after cessation of the progesterone administration. He agreed with Hillarp in his description of a group of persistent diestrous animals, and observed that these animals did not resume normal cycles after progesterone treatment.

Through the work of Donovan and Harris (1954) it was shown that functional hypophysial portal vessels are necessary if the pituitary gland is to maintain a normal estrous cycle. It is probably through these vessels then that neuro-humoral substances are transmitted to the pituitary from the hypothalamus.

If the hypothalamus does control the secretion of the gonadotrophic hormones as the foregoing work indicates, questions then arise as to the exact nature of this control. Is the influence of the hypothalamus limited to control of hormone release from the gonadotrophic cells or is control of hormone synthesis also involved? If the control is through either of these mechanisms and does not involve both to the same degree, then the pituitary of the lesioned animals should show some change in content of gonadotrophins over that of normal glands. It was on this basis that assays of FSH and LH content of pituitaries of lesion-bearing animals were made. It was expected that these assays would give some insight into the mechanism of alteration of the estrous cycles.

In much of the above work, emphasis has been placed on the constant estrous type of alteration of the estrous cycle with only casual mention of the persistent diestrous type animals. This is probably due to the fact that in previous work these animals have represented only a small percentage of all lesioned animals and that these animals are less striking in their differences from irregular cycling animals. But with some criteria of separation of

these persistent diestrous animals it would seem that they would be as informative as the constant estrous animals in the investigation of the areas of the hypothalamus involved in the neural control of the reproductive function.

Furthermore, special efforts were made to note any gonadal atrophy since Greer (1958) stated that no ovarian or uterine atrophy had been reported in the female rat bearing hypothalamic lesions. It was thought that the possibility of finding such atrophy in the female rat would be increased by a large series of lesioned animals and by the production of more extensive lesions.

With the above considerations a correlation of vaginal smear records, location of lesions, organ weight studies, pituitary cytological studies, and ovarian histology of all lesioned animals, combined with other studies such as fertility and pseudopregnancy tests, was made. It was thought that such a method would result in a more complete interpretation of the effects of hypothalamic lesions, especially in regard to reproductive function.

Materials and Methods

I. *Lesions*

A total of two hundred and fifty 26-day old, female, Holtzman rats, divided into four series, were used in this study. The animals were anesthetized with nembutal (4 mg./100 gm. body weight) injected intraperitoneally. Animals were placed in the Labtronics stereotaxic instrument, modified for the head of the rat. The scalp was incised in the midline and small burr holes were made in the skull on either side of the midline at the appropriate place with a dental drill. The dura was pierced with a sharp needle and electrodes were placed bilaterally in the hypothalamus. All lesions were made 1.0 mm. above the floor of the skull and 4.5 or 5.0 mm. anterior to the anterior-posterior zero (AP O) plane, the plane through the external auditory meatus. Electrodes were made from 20 gauge Nichrome wire, and from 25 to 30 gauge stainless steel wire. They were insulated with Epoxylite electrode varnish except at the tip. Lesions of different size were made by varying the electrode size and the milliamperage and duration of the electrolytic lesion current applied. Following the application of the current, the electrodes were withdrawn and skin clips placed in the scalp.

The animals were placed in the animal room, which was kept at a constant temperature of 80° F., and fed Purina laboratory chow and tap water. Some groups were given 5% sucrose solution and canned cat food for short periods post-operatively in an attempt to decrease mortality.

II. *Smear Records*

Daily vaginal smear records were kept on all animals. The smears were started at about 40 days of age and continued until the animals were sacrificed.

III. *Autopsy and Histological Studies*

The majority of the animals were sacrificed at 60 days of age. One series used in the fertility tests were sacrificed at 100 days. The animals were decapitated and the organs removed, cleaned, weighed on a torsion balance, and fixed. Ovaries, uteri, and thyroid, adrenal, and preputial glands were fixed in Bouin's fixative. Six-micron paraffin sections were prepared and stained with H&E for routine histological studies. Pituitaries, of animals not used for assay, were fixed in 10% formol-saline and 4-micron, serial sections were made. Sections from ¼, ½, and ¾ levels of each paraffin ribbon were mounted and stained with PAS-methyl blue or Aldehyde Fuchsin (Rennels, 1957).

The brains were fixed in 10% neutral formalin, trimmed, and the hypothalamus as a block embedded in paraffin. Serial, 10-micron sections were made and stained with cresyl echt violet. The lesions were plotted on an atlas for each animal.

For localization of lesions an atlas of the 60-day old rat hypothalamus was necessary. Several animals of this age were placed in the stereotaxic instrument and bilateral needle tracts made at two different anterior-posterior levels 1 mm. apart. The brains were fixed immediately and embedded in paraffin. Ten-micron serial sections were prepared and stained. The needle tracts were located and the number of sections between needle tracts counted. Using this number to equal 1 mm. sections representing every .5 mm. coordinate were marked. These sections were then projected and outline tracings were made showing the larger nuclei and fiber tracts. Mimeographed copies of these tracings were used in localizing lesions. The lesion sections were projected on the tracing and the lesioned area marked.

IV. *Assay Method*

A. Collection of pituitary powder.

Animals to be assayed were divided into the following groups on the basis of smear records: (1) regular cycles, (2) persistent diestrus, and (3) constant estrus. At autopsy the pituitaries were removed and the posterior lobe separated. The anterior lobe was then weighed and placed between two clean glass slides. The slides were compressed and then separated and placed in desiccators in 37° C. oven. When completely dried the slides were then scraped with a razor blade and the powder collected on Glassine weighing paper. The powder was transferred with the aid of a camel hair brush to glass vials and this material was kept under refrigeration until used.

B. LH Assay (Anterior Prostate Method-Greep, 1941)

Male, weanling Holtzman rats (21 days old) were used for this assay. They were hypophysectomized and maintained on 5% sucrose solution and Purina laboratory chow. The pituitary powder to be assayed was suspended in distilled water such that 1 cc. carried 1.9 mg. of dry powder. The assay material was injected subcutaneously once a day beginning two days after hypophysectomy and continued for 4 days, each assay animal receiving a total dose of 0.75 mg. of dry pituitary powder. Twenty-four hours after the last injection the animals were sacrificed. The anterior prostate, adrenals, and testes were removed and weighed on a torsion balance. The adrenal weight was used as an indication of completeness of hypophysectomy. The sella tur-

cica was also examined for any fragments of pituitary. Only data from animals with adrenals weighing less than 13.5 mg. were included.

C. FSH Assay (HCG Augmentation Method—Steelman and Pohley, 1953)

Assay animals were intact, 21-day old female, Holtzman rats. The pituitary powder to be assayed was suspended in distilled water such that 1 cc. carried 3.3 mg. of dry powder. Human Chorionic Gonadotrophin (Lot #222158, Parke, Davis and Co.) was suspended in distilled water such that 1 cc. carried 100 I.U. The pituitary powder was injected subcutaneously once a day for 3 days with injection of HCG intraperitoneally at the same time, each assay animal receiving a total of 30 I.U. of HCG and 1 mg. of pituitary powder. Animals were sacrificed 24 hours after the last injections. The ovaries and uteri were removed, cleaned, and weighed. In this assay FSH activity is measured by augmentation of ovarian weight over that of HCG alone.

V. *Fertility Tests*

Animals of one group of lesioned animals were placed with adult males on the day of proestrus as determined by smear records and kept with the males for 4 days. Continuous smear records were kept on these animals. If pregnancy did not result, the animals were again placed with males on the next proestrous day.

VI. *Uterine traumatization*

The uteri of four persistent diestrous animals and four constant estrous animals were traumatized. The animals were anesthetized with ether and the uteri were exteriorized through a ventral midline incision. A wire with a hooked tip was then inserted into an incision near the uterine tube and by passing the tip to the cervix and back the endometrium was scratched along the antimesometrial border. The animals were sacrificed 7 days later and the uteri examined for decidual reaction.

Results

1. *Smear Records*

The daily vaginal smear records of all lesioned animals can be classified into four groups. One group of animals had a smear record of regular four to five day estrous cycles. These cycles were identical to those of non-lesioned, control animals. A second group had a smear record which was characterized by a constant cornified smear, the constant estrous group. A third group was characterized by long periods of smears containing only leucocytes; persistent diestrus. The fourth group had smear records which showed irregular estrous periods of varying length.

For simplicity of results all lesioned animals will be grouped according to the above classifications. In order to separate irregular cycling animals from persistent diestrous animals an arbitrary division was made. Any animal with long periods of

diestrus smears but with more than one estrous (cornified) smear during the three weeks of smear records was grouped with irregular cycling animals. Animals with only one estrous smear were grouped with the persistent diestrous animals.

One other group which will be used contains the anestrus animals. Only one lesioned animal had failure of vaginal opening, but on the basis of organ weights one other animal will also be included in this group. This animal had constant diestrous smears from the time of vaginal opening.

II. *Body and Organ Weights*

Organ weights and body weights obtained at autopsy are presented in Table I. Body weights are in grams; all other weights are in milligrams. The organ weights are absolute and in comparing certain groups statistical analyses were made using the "t" test for significance.

The two anestrous animals listed at the bottom of Table 1 show the most marked differences in organ weights. All organ weights of these animals are reduced to near hypophysectomy levels. The animals themselves were much larger than the control animals, being very obese. In the following analysis of results these anestrous animals are excluded from consideration.

The body weights of all lesioned animals show more variability than do the control body weights. This is due to the distribution throughout all lesioned groups of obese animals.

The anterior pituitary weight of all lesioned animals is significantly lower than that of the control animals. Also, the pituitary weight of the persistent diestrous animals is seen to be significantly lower than that of the constant estrous animals.

The ovaries of the constant estrous animals were much smaller than those of control animals. Also, persistent diestrous animals had ovaries which were significantly smaller than control ovaries. The regular and irregular cycling animals showed reduced ovarian weights but when compared to the controls the differences were not significant statistically.

The uteri of all groups were extremely variable in weight but none of the lesioned groups showed significant difference from the uteri of the controls.

The adrenals of all lesioned animals were significantly smaller

TABLE 1

Autopsy Data

(All Animals 60 Days Old)

Group	Body Wt gm.	Anterior Pituitary mg.	Ovaries mg.	Uteri mg.	Adrenals mg.	Thyroid mg.	Preputial Glands mg.
Non-lesioned Control	185 ± 0.6* (31)**	8.35 ± .17 (31)	71.6 ± 3.1 (12)	389 ± 28 (12)	53.3 ± 1.6 (12)	11.9 ± 0.5 (12)	101.6 ± 6.5 (12)
Constant Estrus	173 ± 6.0 (26)	7.56 ± .37 (15)	29.7 ± 1.0 (26)	397 ± 21 (19)	37.6 ± 1.5 (28)	12.7 ± 0.8 (19)	77.0 ± 5.7 (19)
Persistent Diestrus	174 ± 5.3 (28)	6.12 ± .20 (24)	60.1 ± 2.1 (28)	365 ± 45 (11)	38.1 ± 1.5 (27)	11.0 ± 0.4 (11)	75.7 ± 6.3 (11)
Regular Cycles	182 ± 4.2 (53)	7.10 ± .19 (34)	64.2 ± 2.0 (53)	354 ± 14 (27)	43.2 ± 1.2 (52)	12.3 ± 0.3 (26)	80.8 ± 3.4 (27)
Irregular Cycles	180 ± 6.6 (20)	6.56 ± .19 (18)	57.7 ± 2.9 (20)	295 ± 24 (5)	40.7 ± 1.5 (20)	12.7 ± 0.7 (5)	83.8 ± 17.5 (5)
Anestrus	242 ± 8.0 (2)	....	7.3 ± 2.3 (2)	41.5 ± 1.2 (2)	16.8 ± 0.6 (2)	6.4 ± 0.6 (2)	44.9 ± 3.1 (2)
Statistics (P values)		1:2 < .001 2:3 < .001	1:3 < .01 1:4 > .05		1:2 < .001 1:4 < .001	1:2 > .4	1:4 < .01

* Standard Error.
** Number of Animals in Group.

than those of control animals. However, no significant differences were seen between groups of lesioned animals.

The thyroids of all lesioned animals were not significantly different from those of control animals.

The preputial glands of all lesioned animals were significantly smaller than those of control animals again with no significant difference between the various lesioned groups.

III. *Histological Results*

A. Localization of Lesions

The lesions which produced the anestrus animals were very extensive lesions destroying nearly all of the hypothalamus. They extended far forward destroying the anterior hypothalamic area and anterior tip of the arcuate nucleus. They also destroyed major portions of the filiformis nucleus (nucleus paraventricularis), dorsomedial and ventromedial nuclei, arcuate nucleus and tuberal regions. The lesions extended posteriorly to the mammillary body destroying portions but not all of the posterior hypothalamic area (Fig. 1).

Two types of lesions were found to produce a constant estrous state. One type which represented the majority of these lesions was fairly large with anterior placement. These lesions destroyed major portions of the anterior hypothalamic area and anterior part of the arcuate nucleus. This type lesion also destroyed the filiformis nucleus but left major portions of the dorsomedial and ventromedial nuclei intact. They extended nearly to the floor of the hypothalamus anteriorly (Fig. 2). The other lesion resulting in a constant estrous state is a very small bilateral lesion extending over an area of about ½ mm. The lesion extends to the floor just lateral to the arcuate nucleus (at level A 5.0 mm. anterior to

EXPLANATION OF FIGURES

Abbreviations used in figures 1–6.

Ant. — Anterior Hypothalamic Area
Arc. — Arcuate Nucleus
D M — Dorsomedial Nucleus
Fil — Filiformis Nucleus (Paraventricular Nucleus)
Lat. — Lateral Hypothalamic Area
M. T. T. — Mammillothalamic Tract
S. O. — Supra Optic Nucleus
V M — Ventromedial Nucleus
Z I — Zona Inserta

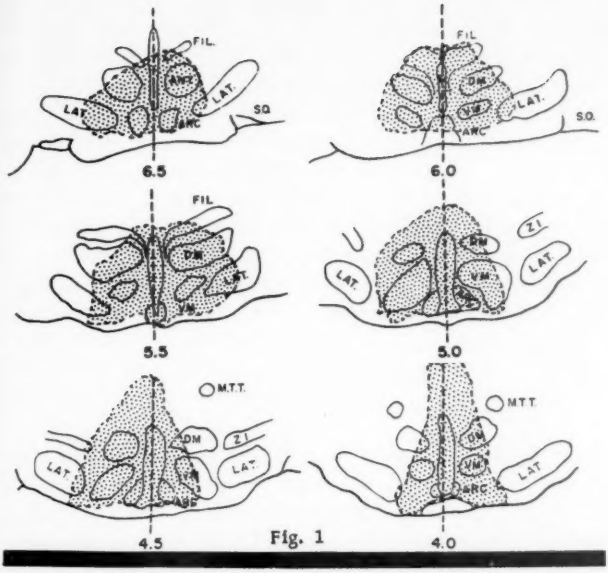

Fig. 1

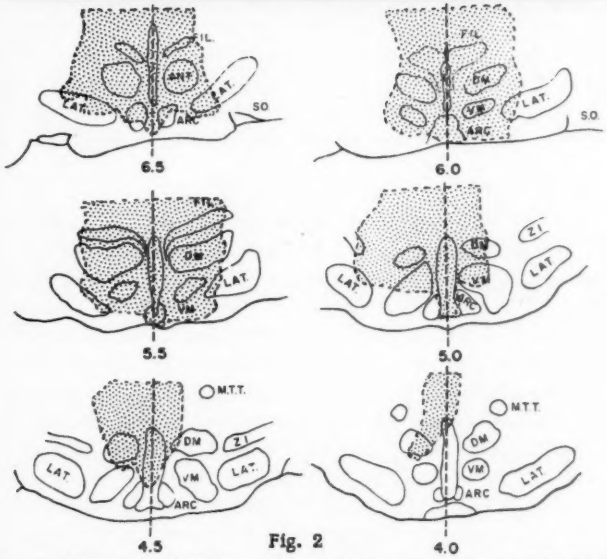

Fig. 2

Fig. 1. Hypothalamic lesion of one of the two anestrous animals. Stippled areas were destroyed by the lesion. Note the very extensive nature of the lesion which destroyed the major portion of the hypothalamus.

Fig. 2. Hypothalamic lesion of an animal in constant estrus. This is a typical, large, anterior type lesion which resulted in constant estrus.

AP O plane). It also involves a very small portion of the ventromedial nucleus just above this area (Fig. 3).

Lesions resulting in persistent diestrous animals were more superior lesions at about the same level as the large constant estrous lesions. The lesions destroyed the filiformis nucleus, anterior portions of the dorsomedial nucleus and the periventricular gray matter medial to the dorsomedial nucleus. These lesions did not extend to the floor and left major portions of the arcuate nucleus and the tuberal region intact. They did not extend as far posteriorly leaving all of the posterior arcuate nucleus and the dorsomedial and ventromedial nuclei intact (Fig. 4). The smallest lesion producing persistent diestrus destroyed the dorsomedial nucleus and the area just superior to it only at level A 5.0 (Fig. 5).

The majority of lesions resulting in normal cycling animals or irregular cycling animals were either asymmetrical, unilateral, or were in the thalamus not reaching the hypothalamus. Some fairly extensive lesions of the hypothalamus, however, did not result in alteration of cycles. One of the most extensive of these lesions involved large parts of the anterior hypothalamic area, the dorsomedial and ventromedial nuclei, and the filiformis nucleus. Only partial destruction of the arcuate and tuberal regions occurred. This lesion did not extend very far superiorly but did extend posteriorly to the mammillary body (Fig. 6).

B. Ovaries

The ovaries of the various groups were very characteristic. The most marked difference from normal ovaries was seen in the ovaries of the anestrous group. The ovaries were atrophic with no Graffian follicles present. There were only a very few well developed primary follicles. No corpora lutea were present and the interstitial tissue was poorly developed (Fig. 7).

The ovaries of the constant estrous animals, although grossly much smaller than the normal ovaries, had numerous very large Graffian follicles. The granulosa layer of these follicles showed marked proliferation, it was many cell layers thicker than normal and was thrown into folds. The ova of many of these follicles showed degenerative changes. The interstitial tissue was well developed and close examination of the cells showed no clumping of nuclear chromatin characteristic of inactive cells. The most

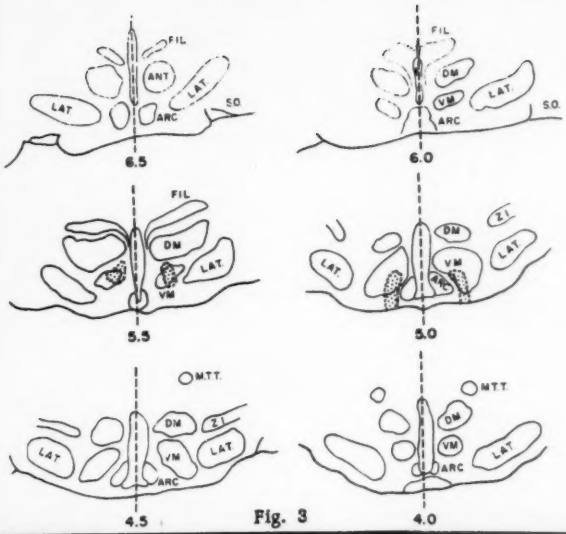

Fig. 3

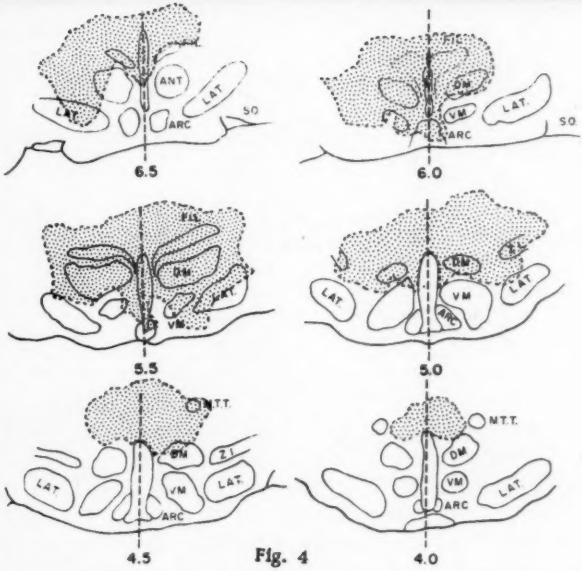

Fig. 4

Fig. 3. Hypothalamic lesion of an animal in constant estrus. This is the small, bilateral type lesion which destroyed only portions of ventromedial nucleus and extended to the floor lateral to the arcuate nucleus.

Fig. 4. Hypothalamic lesion of an animal in persistent diestrus. Note superior placement of the lesion.

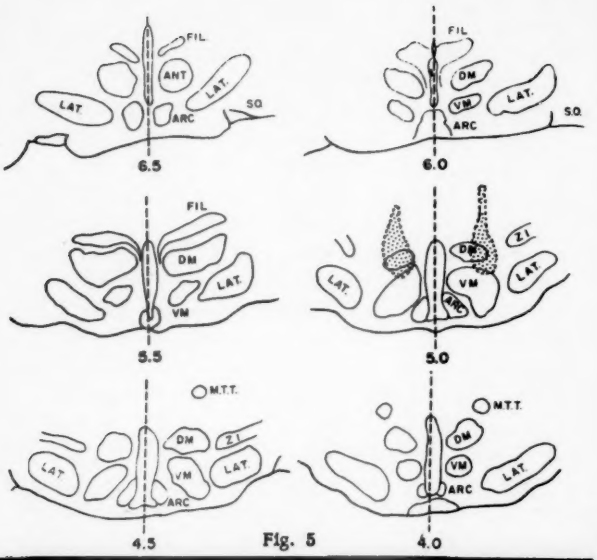

Fig. 5

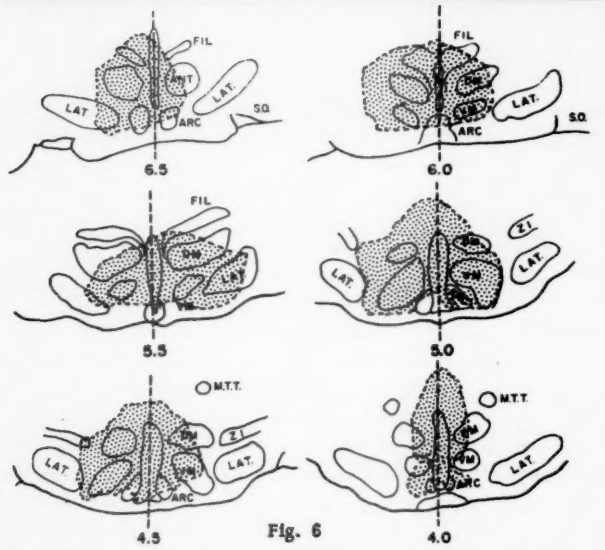

Fig. 6

Fig. 5. Hypothalamic lesion of an animal in persistent diestrus. This was a very small lesion which destroyed a portion of dorsomedial nucleus and the area superior to this nucleus at level A 5.0 only.

Fig. 6. Hypothalamic lesion of an animal which exhibited normal estrous cycles. This is one of the numerous, large, bilateral lesions which resulted in no alteration of the estrous cycle under normal circumstances.

EXPLANATION OF FIGURES 7–10

These figures are photomicrographs of routine H&E, 6 micron sections made at magnification of 25X.

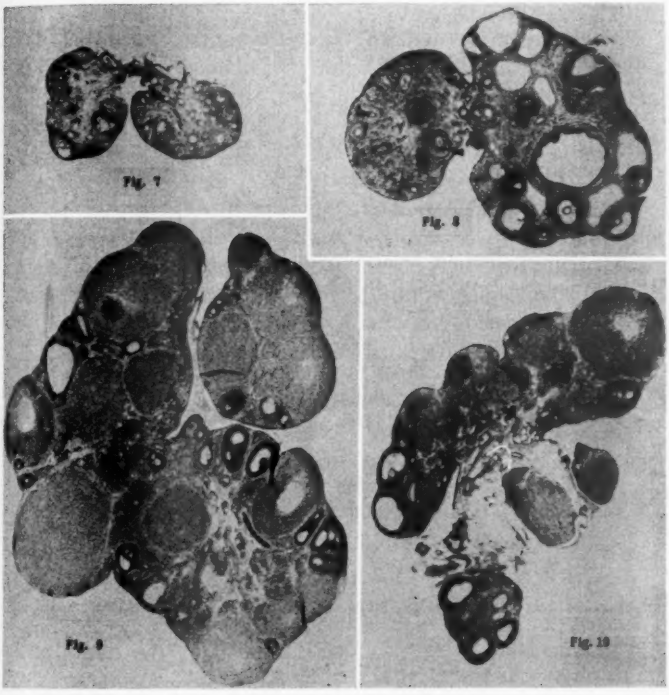

Fig. 7. Ovary of animal in anestrus. Except for the light staining interstitial tissue present, this ovary is typical of that seen in the hypophysectomized animal.

Fig. 8. Ovary of an animal in constant estrus. Note the absence of corpora lutea, and the presence of follicles with well developed stratum granulosum.

Fig. 9. Ovary of an animal in persistent diestrus. Note numerous large corpora lutea and the paucity of medium and large follicles.

Fig. 10. Ovary of a lesioned animal with regular cycles. Although section is tangential in part all structural features are characteristic of a normal ovary.

obvious feature of these ovaries was the complete absence of corpora lutea (Fig. 8).

The ovaries of the persistent diestrous animals, although more variable than those of the constant estrous or anestrous animals, were characterized by numerous very large corpora lutea. The

luteal cells appeared healthy and active. Interstitial tissue was present in normal amounts and showed normal to hyperactive stimulation. Follicles were few in number and with only small antrum formation (Fig. 9).

The ovaries of the lesioned, regular cyclic and irregular cyclic animals did not differ appreciably from those of normal non-lesioned animals (Fig. 10).

C. Pituitary Glands

The anterior lobe of the pituitary gland when examined histologically appeared healthy and well vascularized and no areas of necrosis were seen. Thyrotrophs, red and purple gonadotrophs, acidophiles and chromophobes were all present. Although the glands varied as to numbers of different cell types present, there was no consistent alteration of cytology which could be correlated with alterations in the estrous cycle.

IV. *Assay Results*

A. LH Assay

Results of this assay are presented in Table 2. Four pituitary powders were assayed:

TABLE 2

LH Assay Data

Assay Animal Group**	No.	Body Weight gm.	Ventral Prostrate mg	Testes mg.	Adrenals mg.
Hypex. Control	5	62 ± 2*	11.8 ± 0.8	207 ± 15	12.20 ± .32
Normal Pituitary	5	65 ± 2	20.5 ± 2.1	241 ± 19	11.56 ± .62
Lesioned Normal Cycles Pituitary	6	68 ± 2	18.7 ± 1.5	242 ± 11	11.44 ± .79
Lesioned Constant Estrus Pituitary	4	65 ± 2	20.9 ± 2.6	251 ± 18	10.84 ± .33
Lesioned Persistent Diestrus Pituitary	6	67 ± 2	22.9 ± 2.0	264 ± 55	10.85 ± .62

* Standard Error.
** Assay animals were 22-day-old hypophysectomized, male rats.

1. Powder pooled from non-lesioned, normal cycling animals.
2. Powder pooled from lesioned animals with regular cycles.

3. Powder pooled from lesioned animals in persistent diestrus.
4. Powder pooled from lesioned animals in constant estrus.

Dosages were prepared such that all animals, except hypophysectomized controls, received a total of 0.75 mg. dry weight of pituitary powder over the 4-day injection schedule. Organ weights are absolute and results were analyzed statistically.

There is no statistically significant difference in the organ weights of the injected assay animals but the prostate weight of all injected animals shows an increase over the prostate weight of the uninjected controls. This indicates that there is no significant difference in content of LH of the pituitaries of the lesioned animals as compared to the control animals.

TABLE 3

FSH Assay Data

Assay Animal Group**	No.	Body Weight gm.	Ovaries mg.	Uteri mg.
Uninjected	5	58 ± 2	17.4 ± 0.5	35 ± 1
HCG	6	58 ± 1	33.5 ± 1.9	180 ± 18
HCG + Normal Pituitary	5	61 ± 1	33.6 ± 1.5	127 ± 5
HCG + Lesioned Normal Cyclic Pituitary	4	58 ± 2	34.4 ± 3.0	128 ± 15
HCG + Lesioned Constant Estrus Pituitary	5	58 ± 2	31.1 ± 1.4	131 ± 16
HCG + Lesioned Persistent Diestrus Pituitary	5	59 ± 2	32.8 ± 2.1	130 ± 9

* Standard Error.
** Assay animals were 21-day-old intact, female rats.

B. FSH Assay

Results are presented in Table 3. Six groups of assay animals were used. The same four pituitary powders as above were assayed. The groups used were:

1. Uninjected controls.
2. Animals receiving only HCG.
3. Animals receiving HCG and pituitary powder from normal, non-lesioned animals.
4. Animals receiving HCG and pituitary powder from lesioned, constant estrous animals.

5. Animals receiving HCG and pituitary powder from lesioned, persistent diestrous animals.

6. Animals receiving HCG and pituitary powder from lesioned, regular cycling animals.

Dosages were prepared such that all animals received 30 I.U. of HCG and those receiving pituitary powder had a total dosage of 1.0 mg. of dry pituitary powder. Organ weights are absolute and were analyzed statistically.

The ovaries of the groups receiving HCG and pituitary powder are not significantly larger than those of groups receiving only HCG. This indicates that the dosage of pituitary powder was insufficient to give an indication of FSH activity, although the dose used was similar to that used by Steelman and Pohley (1953) in their assay of pituitary material for FSH. However, this does indicate that there is no marked increase in content of FSH of the pituitaries of lesioned animals as compared to the control animals.

V. *Fertility Tests*

One group of lesioned animals showed only a few animals with altered cycles. It was in this group that tests were made as to fertility. Many of these animals with regular cycles when placed with males would show sperm in the smear and would become pregnant. But litters were small with numerous stillbirths. Other animals would show no sperm in the smear and would not become pregnant after repeated placement with males. It is not known whether these animals would copulate with males or not, but absence of sperm in the smears would suggest that they did not. Two animals, which had had regular cycles before being placed with males, were put into cages with males on the day of proestrus. These animals had completely cornified smears the next day, and the smear remained cornified (constant estrus) until the animals were sacrificed. Three animals developed diestrous smears after being placed with males and were thought to be pregnant, but after 21 days they had not delivered and were sacrificed and found not to be pregnant. None of the animals with persistent diestrous or constant estrous smears became pregnant.

The lesions of this group were very small and the majority were not bilateral or symmetrical.

VI. *Pseudopregnancy Tests*

Of the four persistent diestrous animals which underwent uterine traumatization, three showed no decidua formation after 8 days. One animal of this group died on the day after the operation. The four constant estrous animals which were traumatized also showed no decidua formation at autopsy 8 days later.

Discussion

The estrous cycle in the female is an expression of the cyclic activity of the ovary. Alterations of the normal estrous cycle have been shown to occur because of upsetting any of the interactions of the ovarian hormones and the pituitary gonadotrophins. Everett (1943), after studying a strain of spontaneous constant estrous rats, concluded that this alteration was due to a decreased LH secretion. Hisaw (1947), in his review of follicular development and ovulation, pointed out that certain quantitative amounts of FSH and LH, acting synergistically, were optimal for ovulation and that ovulation could be prevented by upsets of this balance. Thus in Everett's strain of constant estrous rats the optimal LH and FSH relationship for ovulation was never obtained. Another alteration of normal cycles is produced by sterile matings and electrical cervical stimulation. Such stimulation has been shown to increase the diestrous phase of the cycle. This so-called pseudopregnancy is the result of the progesterone produced by the corpora lutea and this secretion is, in turn, dependent on pituitary luteotrophin release. In such animals a positive decidual reaction can be obtained following uterine traumatization.

The hypothalamus has been shown to be related to the secretion of many of the anterior pituitary hormones, although the relationship has been shown more clearly in the case of ACTH, TSH, and the gonadotrophins. Of all pituitary hormones the secretion of luteotrophin (LTH, Prolactin) probably is the least dependent on the hypothalamus. Everett (1954) obtained evidence in rats of luteotrophin secretion from pituitary autotransplants under the kidney capsule and thus relatively free of hypothalamic control. The evidence for the control of somatotrophin (STH) secretion by the hypothalamus has been meager, although Bogdanove and Lepner (1952) showed that animals with

hypothalamic lesions frequently fail to grow even when they become very obese. Although the rats in the present study showed no marked decrease in growth, nor has such a decrease been reported in other work with hypothalamic lesions, further studies of the hypothalamic-STH relationship are needed.

Probably, of all the pituitary hormones studied in relation to hypothalamic control, ACTH and TSH have received the most emphasis. Lesions of the hypothalamus in various positions have been shown by many workers to interfere with some portion of TSH or ACTH activity. Atrophy of the adrenals and thyroid to hypophysectomy levels has been reported previously only by Bodganove (1955), and then only in 4 animals. In the present study only two animals with complete atrophy were seen and these animals had very extensive lesions. In contrast to the high frequency of adrenal and thyroid atrophy following transplantation of the pituitary to sites other than the sella turcica (Harris and Jacobson, 1952), the paucity of reported cases of such atrophy following hypothalamic lesions may be due to a high mortality in animals with extensive hypothalamic lesions, *i.e.*, lesions sufficiently large to result in such atrophy may be approaching the maximum size compatible with the life of the animals. If more rigorous postoperative care of lesioned animals were instituted then more cases of complete adrenal cortical and thyroid atrophy might be reported.

The adrenals of all groups of lesioned animals were seen to be reduced in weight when compared to those of the control animals. Correlated with the decrease in the weight of the preputial glands this would indicate a decrease in ACTH secretion. Jacot and Selye (1952) reported that ACTH was capable of promoting preputial gland growth in the absence of adrenals and ovaries, thus indicating that this effect was a direct one. A reduction in adrenal weight has not been a consistent finding in other studies involving hypothalamic lesions as it was in the present one. In the present study, all animals were operated on while immature, and the hypothalamic lesions were thus present during the period of most active growth of the animals, whereas in other studies the lesions have been placed in mature rats where a small decrease in ACTH secretion might not be sufficient to result in adrenal atrophy. However, a small decrease in the rapidly growing rat might be critical in retarding the normal increase in

adrenal size. The failure of compensatory hypertrophy after unilateral adrenalectomy in mature rats with hypothalamic lesions (Fulford, 1955) would tend to support this assumption.

An excellent review of the relationship of FSH and LH and the hypothalamus has been given by Benoit and Assenmacher (1955). Reviewing the work of numerous investigators on different species of animals, they correlated special areas of the hypothalamus with FSH and LH secretion and inhibition. The majority of the reports covered by this review were studies done on the rabbit where definite hypothalamic areas are known to be concerned with secretion of LH (Markee, *et al*, 1946).

For the rat, the evidence of direct control of FSH and LH is more meager. The majority of workers still agree with Hillarp's conclusion that lesions of the anterior hypothalamus of the rat will block LH secretion resulting in constant estrus. However, one other possibility should be considered, namely that this is due to an increased release of FSH. The marked proliferation of the stratum granulosum in the ovaries of constant estrous animals (Fig. 8) indicated that this may be the case, as suggested by Everett (1952). Either of these two states, decreased LH or increased FSH secretion, would alter the optimal quantitative relationship of LH and FSH, thereby preventing ovulation. The resumption of normal cycles in lesioned constant estrous animals following progesterone administration was concluded to be the result of an increase of LH secretion (Greer, 1958). If either of the above alterations is the true mechanism of production of the constant estrous state, then an increase in LH secretion could cause resumption of cycles. In the case of ovulation failure due to a decrease in LH, then progesterone causing an increase in LH secretion would create the optimal level and ovulation would occur. In the case of ovulation failure due to an increase of FSH, then the increase of LH secretion would create a balance between these two at a level higher than normal, but again optimal for ovulation.

In considering the results of the assays of the pituitaries one must consider both of the above possibilities. The LH content of the pituitaries of the constant estrous animals was not significantly different from that of the non-lesioned animals. If the constant estrous state results from a decreased secretion of LH then the mechanism must be one of a decreased production as

well as a decreased release of this hormone. In this case the actual content might remain unchanged. However, if constant estrus results from normal LH secretion and an increase in FSH secretion, then again there would be no change in the pituitary content of LH. The FSH assays, although inconclusive, showed no marked increase of FSH content of the pituitaries of the constant estrous animals, but also did not preclude a possible decreased FSH content, as compared to the control animals. Therefore, if constant estrous results from an increased release of FSH, it may be effected through an increased secretion with or without a marked increase in production. The absence of marked cytological change in the pituitary of these animals suggests that any increased FSH release is accompanied by a compensatory increase of FSH synthesis. It is generally recognized that any marked change in pituitary content of gonadotrophin is correlated with marked cytological changes in the gonadotrophic cells (Pearse, 1952).

The mechanism of production of the persistent diestrous state seen in this study remains obscure. The pituitaries of these animals, like those of the constant estrous animals, showed no change in their content of LH and no marked increase in FSH content. Because of the negative pseudopregnancy tests observed in these animals, it would seem that the apparently normal corpora lutea present in the ovaries of the persistent diestrous animals (Fig. 9) are not secreting progesterone. This would indicate a deficiency of LTH and a difference in this state of diestrus and that of pseudopregnancy produced by sterile mating or pituitary transplants. The ovaries of these animals also show evidence of a decreased FSH secretion and normal or perhaps greater than normal LH secretion. It is felt that the persistent diestrous state is probably due to the interruption of the secretion of FSH, although the possibility of an altered secretion of LTH must be kept in mind.

All lesioned animals had pituitaries which were much smaller than those of normal animals. No adequate explanation of this difference is available since no areas of necrosis were seen in any of the glands. However, since the assays were run on equal weights of pituitary powder it may be concluded that the total gonadotrophin content on a per gland basis was reduced. This

total decrease of gonadotrophin content is probably responsible for the decreased ovarian weight of all lesioned groups, regardless of estrous cycles. Since evidence of reduced ACTH levels in these glands was also observed, there is a possibility that a panhypopituitarism may exist.

The observation of lesioned, normal cycling animals becoming either persistent diestrus or constant estrus after being placed with males has not been reported before. The lesions of these animals were usually unilateral or asymmetrical in the anterior hypothalamus, not involving any specific areas. Here a definite problem of interpretation of results arises. Although it seems that the areas destroyed were not of sufficient size or extent to alter the cycles under normal circumstances, the added stimulus of males present was sufficient to upset delicate balances and cause alteration of the estrous cycles. Not enough is known as to what this stimulus might produce to draw any more definite conclusions.

The very extensive lesions seen in the anestrous animals in this study (Fig. 1) preclude the definition of areas or nuclei related to specific hormones, but it can be stated that the hypothalamus is essential for the normal functioning of the reproductive system. Also, from these animals it is seen that the hypothalamus is essential for the maintenance of normal adrenal and thyroid weight.

Fairly well localized lesions which result in a diestrous state have been described in this study. These lesions were superior lesions destroying the filiformis nucleus, anterior portions of the dorsomedial nucleus, and the periventricular grey matter medial to the dorsomedial nucleus (Fig. 4). However, with the lack of any definite evidence of the endocrine state of these animals, particularly in regard to LTH and gonadal hormones, it is difficult to draw any conclusions as to the function of this center. The meager evidence available, however, suggests that this area may mediate FSH secretion.

In the rat the only complete agreement so far, has been that lesions of the anterior hypothalamus destroying the major portion of the arcuate nucleus, and small lesions lateral to the arcuate nucleus (Figs. 2 and 3) will result in the production of a constant estrous state. The usual interpretation is that this is

the result of decreased LH production caused by destruction of a center which facilitates LH secretion. The results of this study suggest as an alternative that this hypothalamic area is one which may cause inhibition of FSH secretion.

Finally, a major difficulty in this type of study is the placement of precise lesions. Thus, large numbers of animals are necessary to obtain an adequate number with the desired type of functional alteration. The present study has covered some two hundred and fifty animals and certain types of alteration in gonadotrophic function, that have not received attention in previous reports, have been seen and studied. Many questions still remain to be answered on the relationship of the hypothalamus and the pituitary. Striking for its absence is any evidence as to the state of LTH secretion in the lesioned animals. In future investigation of the mechanisms of hypothalamic control over pituitary function, blood assays of the anterior pituitary hormones in animals with hypothalamic lesions would be most beneficial.

Summary

Four series of female rats, totaling two hundred and fifty animals, bearing bilateral electrolytic lesions in the hypothalamus were studied. By means of organ weight studies a generalized panhypopituitarism was shown to exist in all lesioned animals, especially in regard to ACTH and gonadotrophins. Many of the animals studied were shown to exhibit various alterations of the estrous cycle; *e.g.;* constant estrus, persistent diestrus, and anestrus. Specific areas of the hypothalamus involved in the production of these altered states are described.

The possible mechanisms underlying the production of the altered estrous states are considered. Two possible mechanisms exist for the production of the constant estrous state and these were considered in light of the gonadotrophic assay data obtained. Of the two mechanisms, (1) decreased LH secretion or (2) increased FSH secretion, the latter is considered the more likely explanation for the constant estrous state.

The mechanism of production of persistent diestrus seen in this study was considered to be different from that of pseudopregnancy and presumably the result of inhibition of FSH secretion.

REFERENCES AND NOTES

Bailey, P., and F. Bremer: Experimental diabetes insipidus. Arch. Intern. Med., 28: 773–803, 1921.

Benoit, J., and I. Assenmacher: Le Controle Hypothalamique de l'activité préhypophysaire gonadotrope. J. Physiologie 47: 427–567, 1955.

Bogdanove, E. M., and H. J. Lipner: Intestinal absorption of glucose in hypothalamic obesity. Proc. Soc. Exper. Biol. and Med. 81: 410–412, 1952.

Bogdanove, E. M., and N. S. Halmi: Effects of hypothalamic lesions and subsequent propylthiouracil treatment on pituitary structure and function in the rat. Endocrinology 53: 274–292, 1953.

Bogdanove, E. M., B. N. Spirtos, and N. S. Halmi: Further observations on pituitary structure and function in rats bearing hypothalamic lesions. Endocrinology 57: 302–315, 1955.

Dey, F. L., C. Fisher, C. M. Berry, and S. W. Ranson: Disturbances in reproduction functions caused by hypothalamic lesions in female guinea pigs. Amer. J. of Physiology 129: 39–46, 1940.

Donovan, B. T., and G. W. Harris: Effect of pituitary stalk section on light-induced oestrus in the ferret. Nature 174: 503–504, 1954.

Everett, J. W.: Further studies on relationship of progesterone to ovulation and luteinization in persistent-estrous rat. Endocrinology 32: 285-292. 1943.

————: Presumptive hypothalamic control of spontaneous ovulation Ciba Foundation Colloquia on Endocrinology. Edit. by Wolstenholme, G.E.W., The Blakiston Company, New York, Vol. IV, 167–178, 1952.

————: Luteotrophic function of autografts of the rat hypophysis. Endocrinology 54: 685–690, 1954.

Fulford, B. D., and S. M. McCann: Suppression of adrenal compensatory hypertrophy by hypothalamic lesion. Proc. Soc. Exper. Biol. and Med. 90: 78–80, 1955.

Greep, R. O., H. B. VanDyke, and B. F. Chow: Use of anterior lobe of prostate gland in the assay of metakentrin. Proc. Soc. Exper. Biol. and Med. 46: 644–649, 1941.

Greer, M. A., and H. L. Erwin: Evidence of separate hypothalamic centers controlling corticotrophin and thyrotrophin secretion by the pituitary. Endocrinology 58: 665–670, 1956.

Greer, M. A.: Studies on the influence of the central nervous system on anterior pituitary function. Recent Progress in Hormone Research. Edit. by Pincus, G., Academic Press, Inc., New York, Vol. XIII, 67–104, 1958.

Harris, G. W., and D. Jacobsohn: Functional grafts of the anterior pituitary gland. Proc. Roy. Soc. B. 139: 263–276, 1952.

Harris, G. W.: Neutral Control of the Pituitary Gland. Edward Arnold (publishers) Ltd., London, 1955.

Hillarp, Nils-Ake: Studies on the localization of hypothalamic centers controlling the gonadotrophic function of the hypophysis. Acta Endocrinologica 2: 11–23, 1943.

Hisaw, F. L.: Development of the Graafian follicle and ovulation. Physiological Reviews 27: 95–119, 1947.

Jacot, B., and H. Selye: On extra-adrenal actions of adrenocorticotrophic preparations. Endocrinology 50: 254–258, 1952.

Markee, J. E., C. H. Sawyer, and W. H. Hollinshead: Activation of anterior hypophysis by electrical stimulation in the rabbit. Endocrinology 38: 345–357, 1946.

Pearse, A. G. E.: Cytochemical localization of the protein hormones of the anterior hypophysis. Ciba Foundation Colloquia on Endocrinology. Edit. by Wolstenholme, G. E. W., The Blakiston Company, New York, Vol. IV, 1–19, 1952.

Rennels, E. G.: Two tinctorial types of gonadotrophic cells in the rat hypophysis. Z. Zellforsch. 45: 467–471, 1957.

Steelman, S. L., and F. M. Pohley: Assay of the follicle stimulating hormone based on the augmentation with human chorionic gonadotrophin. Endocrinology 53: 604–616, 1953.

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THE EFFECT OF ARTIFICIALLY INDUCED ASCITES ON THE PRESSURE OF THE PORTAL VEIN

JOHN R. DERRICK*

A frequent complication of portal hypertension is bleeding from areas of venous distension such as esophageal varices. Since the lowering of portal venous pressure in such cases (7) should aid in hemostasis, a knowledge of the causal factors in portal hypertension would be invaluable in the development of effective treatment. One possible causal factor in portal hypertension is the massive ascites often found in patients with hepatic cirrhosis (13).

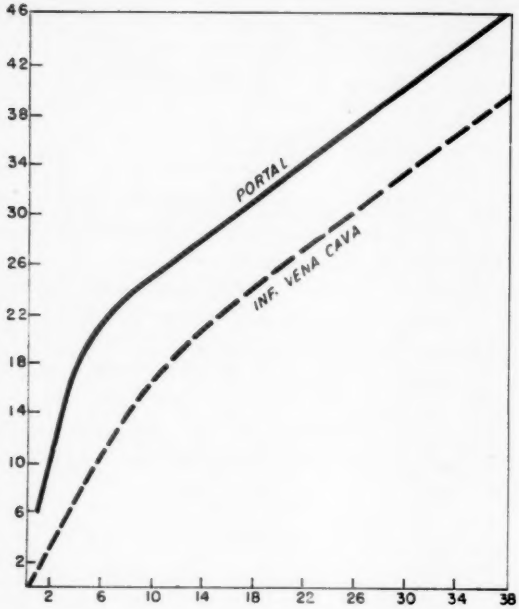
The experiment reported herein was designed to evaluate the effects of experimentally produced ascites on the portal venous pressure.

Method

Ten healthy dogs were anesthetized with sodium pentobarbital to a state of complete muscular relaxation. The abdomen was opened, and heparin (2 milligrams per kilogram of body weight) was administered. Number 18 polyethylene catheters were inserted into the portal vein, the inferior vena cava, and the general peritoneal cavity and, in five dogs, into the femoral vein, and were brought out through separate stab wounds. The primary incision was then closed. Each catheter was connected to its manometer, and control measurements were made. All pressures were measured in centimeters of water. A 6 per cent solution of dextran (average molecular weight approximately 50,000), containing .5 cc. methylene blue dye per 500 cc. dextran, was then gradually infused into the peritoneal cavity. To exclude the possibility of the solution's absorption from the peritoneal cavity, the concentration of methylene blue in the portal venous blood was estimated by photometer at various times during the experiment. No absorption was detected.

Results

The pressures recorded before peritoneal infusion were similar to those reported by Grindlay and associates (10). The initial increment of 6 to 8 cm. H_2O in the intraperitoneal pressure consistently resulted in a precipitous rise in the portal pressure to 20 to 24 cm. H_2O (Figure 1). Subsequent increments in the intra-



THE INFLUENCE OF INTRA-ABDOMINAL PRESSURE UPON THE INFERIOR VENA CAVA AND PORTAL PRESSURES. (PRESSURES RECORDED IN CMS. OF WATER)

peritoneal pressure, after the initial 6 to 8 cm. rise, were reflected in a parallel rise in portal pressure. Pressures in the inferior vena cava, the peritoneal cavity, and the femoral vein tended to be equal throughout the experiment. This same similarity has been reported by Davidson *et al.* (6).

Discussion

That portal pressure is increased by contractions of the diaphragm or abdominal muscles is borne out by many studies. Taylor (16) and Bellis (2) noted that abdominal and respiratory movements in normal patients caused a rise in portal pressure to 50 to 55 cm. H_2O. Similar increases occurred in patients with portal hypertension. Taylor was able to demonstrate a pressure

gradient of 160 cm. H_2O across the diaphragm during forced deep breathing. Alexander (1) reported that diaphragmatic contraction produced by stimulation of the phrenic nerve was accompanied by an increase in portal pressure, despite a reduction of portal inflow from the mesenteric bed.

Hoffbauer and co-workers (12) reported that the straining of urination, defecation and vomiting produced sharp increases in the portal pressure of normal dogs. Ingestion of food was followed by a rise in portal pressure which persisted for several hours. Bradley (4) found that a pressure of 80 mm. Hg. applied externally to the abdominal wall reduced hepatic blood flow in 5 of 8 patients studied. This reduction in hepatic blood flow might have resulted from an increased portal pressure followed by diversion of portal blood through collateral channels, or from a reduction of blood flow through the mesenteric bed.

Although the ascites in our experiments was an artificial one and was produced more rapidly than usual, the conclusion that ascites contributes toward increased portal venous pressure seems justified. Massive ascites, by producing physiological and anatomical derangement (3), compromises renal (8), cardiac, respiratory and digestive efficiency. The findings of the present study and of those concerned with diaphragmatic and abdominal contractions indicate that any increase in intra-abdominal pressure results in a rise of portal pressure.

The effect of arterial blood pressure on portal pressure seems to be much greater in patients with hepatic cirrhosis than in those without this abnormality. (5, 9, 14, 17). Herrick (11), for example, found that elevations in arterial pressure of 100 mm. Hg. or more were required to significantly affect the portal pressure in dogs with normal livers, whereas in those with cirrhotic livers, portal pressure was increased when arterial pressure was raised as little as 30 mm. Hg. Once a rise in portal pressure had been effected, further 1 mm. elevations were produced with increments of 40 mm. Hg. arterial pressure in normal dogs; elevations of only 6 mm. Hg. in arterial pressure were required to produce 1 mm. Hg. increase in the portal pressures of dogs with hepatic cirrhosis. McIndoe (15), however, was unable to verify all of the above data.

McIndoe (15) found that 100 per cent of a fluid perfused

through the portal vein at autopsy could be recovered in the hepatic vein of those with normal livers. If the liver was cirrhotic, however, the greatest amount of the fluid which could be recovered was 13 per cent. He considered this decreased recovery to be a result of the deviation of the perfused fluid through collateral channels which are present in patients with cirrhosis.

Since any rise in intra-abdominal pressure may increase the portal pressure, prevention or relief of high intra-abdominal pressures should prove helpful in treatment of hemorrhage from esophageal varices. Should ascites exist in a patient with such hemorrhage, its relief by abdominal paracentesis may thus be indicated.

REFERENCES AND NOTES

1. Alexander, R. S.: Influence of Diaphragm on Portal Blood Flow and Venous Return. Federation Proc., 9:4, 1950.
2. Bellis, C. J.: The Portal Venous Pressure in Man. Proc. Soc. Exper. Biol. and Med., 50:258, 1942.
3. Bennett, H. D., C. Lorentzen, and L. A. Baker: Transient Esophageal Varices in Hepatic Cirrhosis. Arch. Int. Med., 92:507, 1953.
4. Bradley, S. E.: Studies of Hepatic Blood Flow in Man. J. Clin. Invest., 25:918, 1946.
5. Bradley, S. E., F. J. Ingelfinger, and G. P. Bradley: Hepatic Circulation in Cirrhosis of the Liver. Circulation, 5:419, 1952.
6. Davidson, C. S., T. B. Gibbons, and W. W. Faloon: Systemic and Portal Venous Pressures in Cirrhosis of the Liver. J. Lab. and Clin. Med., 35: 181, 1950.
7. Dye, W. S., H. D. Bennett, L. Baker, and O. C. Julian: Observations on the Inconstancy of Portal Hypertension in Hepatic Cirrhosis. Surgical Forum, 3:496, 1953, W. B. Saunders Company, Philadelphia.
8. French, D. M., P. A. Molano, and W. M. Booker: Renal Function Related to Acutely and Chronically Raised Intra-Abdominal Pressure in Anesthetized and Unanesthetized Dogs. Am. J. Physiol., 167:241, 1951.
9. Friedman, E. W., and R. S. Weiner: Estimation of Hepatic Sinusoid Pressure by Means of Venous Catheters and Estimation of Portal Pressure by Hepatic Vein Catheterization. Am. J. Physiol., 165:527, 1951.
10. Grindlay, J. H., R. Thors, and J. L. Bollman: Effect of Occlusion of Vessels Entering the Liver on Hepatic Venous Pressure in Normal and Cirrhotic Dogs., Surgical Forum, 3:493, 1953, W. B. Saunders Company, Philadelphia.
11. Herrick, F. C.: An Experimental Study Into the Cause of the Increased Portal Pressure in Portal Cirrhosis. J. Exper. Med., 9:93, 1907.
12. Hoffbauer, F. W., J. L. Bollman, and J. H. Grindlay: Factors Influencing Pressure in the Portal Vein as Measured in the Intact Animal. J. Lab. and Clin. Med., 32:1401, 1947.
13. Linton, R. R.: Portal Hypertension. Trans. Seventh Liver Injury Conference, Josiah Macey Jr. Foundation, Page 42, 1948.

14. MacLeod, J. J. R., and R. G. Pearce: The Outflow of Blood from the Liver as Affected by Variations in the Condition of the Portal Vein and Hepatic Artery. Am. J. Physiol., 35: 87, 1914.

15. McIndoe, A. H.: Vascular Lesions of Portal Cirrhosis. Arch. Path., 5: 23, 1928.

16. Taylor, F. W.: Portal Tension and Its Dependence on External Pressure. Ann. Surg., 140: 652, 1954.

17. Wayne, H., J. T. Joyner, W. McCall, and H. D. Green: Influence of Hepatic Blood Flow Upon Resistance to Prolonged Arterial Hypotension. Federation Proc. (Abst.), 10: 143, 1951.

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SMOKE CONDENSATES ON LUNG CELLS IN TISSUE CULTURE WITH SPECIAL REFERENCE TO CHROMOSOMAL CHANGES

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Introduction

Recent studies dealing with the development of cell lines *in vitro* have demonstrated a somewhat unexpected degree of variability in chromosome number and structure. In many reports evidence is presented which leads to the belief that the genesis of changes in the chromosomal complex arises immediately after

explantation. Following upon a phase in which there is a broad variation in chromosome number and structure, during which mitotic activity generally reaches a very low level, a stem line may develop which appears to be relatively stable. The extrinsic factors involved in these events constitute one of the most challenging areas for cytological inquiry.

Established cell strains invite quantitative studies on the effect of chemicals suspected of influencing the chromosomal complex either by altering the position of the stem line number or by producing structural anomalies in statistically significant numbers.

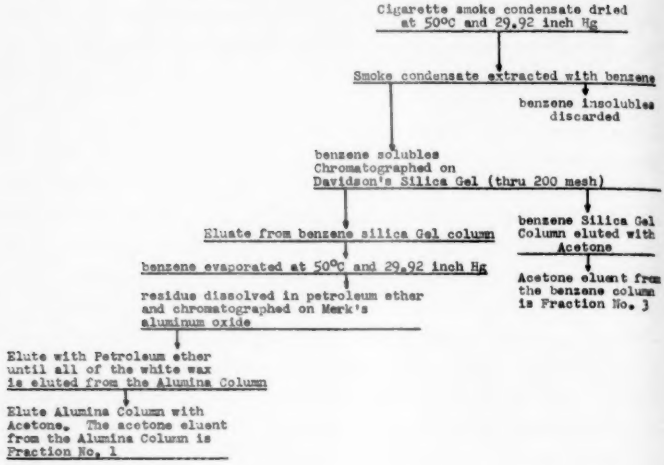
The goals of this paper involved attempts to develop human epithelial lung strains with and without the presence of tobacco tar derivatives. In addition, such substances and benzpyrene were employed to determine whether the chromosomal configuration of an established lung strain could be altered.

Materials and Methods

Lung tissues from a two-and-a-half month male human fetus and from newborn to 4-week-old kittens were used as test materials. Kitten tissue was employed especially for closed chamber experiments. Three explants of about 1 to 1.5 mm. side length were set up on 12 × 50 mm. cover slips of #1 thinness with a clot consisting of equal parts of heparinized rooster plasma and embryonic extract. When the clots were firmly set, the cover slips were placed in roller tubes. For long term cultivation, 40 to 70 explants were set up in culture bottles measuring 1½ x 2½ x 4½ inches with the use of an avian plasma clot. Two to three hours after explantation, Eagle's medium with 10 percent horse serum was added, 2 ml. for roller tubes and 30 ml. for the bottles. The roller tubes were incubated at 37°C. at 8 revolutions per hour. The culture bottles were maintained at the same temperature but without regular movement.

For this experiment two kinds of smoke condensates were used to treat the cells; one was purchased from the Ecusta Paper Company (Code No. 9705), and the other was kindly supplied by Dr. Jack Neal of the Research Laboratory of Industrial Hygiene of this medical school. As presented schematically in text-figure 1, Neal's No. 1 and No. 3 fractions of smoke condensate consisted of benzene extracts of the smoke condensate chromatographed through an alumina column and eluted with petroleum ether (No. 1), or through silica gel and eluted with acetone (No. 3) (26).

Further details of the methods used are described in each section under "Results."


Cigarette smoke condensate dried at 50°C and 29.92 inch Hg
Smoke condensate extracted with benzene
benzene insolubles discarded
benzene solubles Chromatographed on Davidson's Silica Gel (thru 200 mesh)
Eluate from benzene silica Gel column
benzene Silica Gel Column eluted with Acetone
benzene evaporated at 50°C and 29.92 inch Hg
Acetone eluent from the benzene column is Fraction No. 3
residue dissolved in petroleum ether and chromatographed on Merk's aluminum oxide
Elute with Petroleum ether until all of the white wax is eluted from the Alumina Column
Elute Alumina Column with Acetone. The acetone eluent from the Alumina Column is Fraction No. 1

* Prepared by Dr. Jack Neal

TEXT-FIGURE 1

Fractionation of cigarette-smoke condensate

Results

I. *Preliminary Studies on the Effect of a Smoke Condensate on Cells in Primary Culture.*

1. *Morphology of the mitochondria (Perfusion series).*

The changes in the appearance of kitten lung cells in response to the Ecusta smoke condensate were successively followed in single cells in closed chamber preparations which were perfused with smoke condensate at 1:400, 1:800, and 1:1200 diluted in Eagle's nutrient medium containing 10 per cent horse serum. Records made with phase contrast microscopy showed that injury to mitochondria (ring forms) was obtained at a 1:400 dilution within 61 minutes (Figs. 1 to 4). Mitochondria were fragmented when in contact for 137 minutes at a 1:800 dilution and in as little as 28 minutes at a 1:1200 dilution, but such cells were believed to have survived the treatment for at least several hours (Figs. 5–12).

2. *Morphological changes of the cells (Roller tube series).*

a. *Kitten Lung.* Explants of kitten lung on long cover glasses in roller tube cultures were treated with smoke condensate at 1:200, 1:400, 1:600, 1:800, 1:1600, 1:3200, 1:6400, and 1:9600 dilutions in Eagle's medium. Three days later the cultures were fixed with methyl alcohol and stained according to Jacobson's method. Figure 13 represents untreated cells. The nuclei and nucleoli can be clearly seen. Figures 14 to 16 show pycnotic cells at 1:200, 1:400, and 1:800. On the 2nd and 7th days the untreated cultures in closed chambers were observed with phase contrast microscopy. In untreated cells the filamentous mitochondria were easily demonstrated in the cytoplasm (Fig. 17). The cells in contact with 1:400 and 1:600 dilutions showed pycnotic degeneration (Figs. 18, 21), and a few multinucleate elements were observed (Fig. 22). Some damage was found in cultures with as little as 1:3200 and 1:6400 but the cells appeared viable on the 7th day (Figs. 19, 23, 24). At a 1:9600 dilution the appearance of most of the cells was similar to that in control cultures (Fig. 20).

b. *Human lung.* Fibroblast-like cells from human lung tissue in roller tube cultures were treated with the Ecusta smoke condensate at 1:400, 1:800, 1:1600, 1:3200, and 1:10,000 dilutions in Eagle's medium. The cultures were observed with phase contrast microscopy on the 2nd and 7th days after treatment. At the 1:400 and 1:800 dilutions pycnotic degeneration was prevalent (Figs. 26, 27, 32, 33). At a 1:1600 dilution some cells were damaged and the mitochondria showed irregularities in form (Figs. 28, 34). At 1:3200 and 1:10,000 dilutions, however, the cells were not damaged for at least 7 days (Figs. 25, 29, 30, 31, 35, 36).

3. *Chromosomal studies of human lung cells.*

a. *Chromosome counts.* On the basis of the experiments which have been described, it was decided to make a more detailed study of cell injury which might result from long-term contact with the Ecusta smoke condensate at a 1:10,000 dilution in the nutrient fluid employed for the maintenance of human fetal lung in bottle cultures. Chromosome counts were made of smoke condensate treated cells together with corresponding untreated controls. The major part of the chromosome study was carried out

with the use of squash preparations. Cell samples were collected from the bottle cultures with the aid of a rubber policeman and a glass pipette. The cells were treated with a hypotonic solution (1 part of Gey's balanced salt solution and 3 parts of tap water) for 10 minutes and then were stained with acetic orcein for 30 to 60 minutes. Furthermore, the roller tube cultures were employed to study the chromosomes both of control and treated cells. In a series of growing cultures, 0.04 ml. of 1:10,000 colchicine solution in Eagle's medium was added to each roller tube containing 2 ml. of medium and incubated for 16 to 20 hours. At the end of this period the cultures were treated with a hypotonic solution and then fixed with methyl alcohol. Finally, they were stained according to Jacobson's method. A total of over 150 explants was used for this study.

Results are presented in text-figure 2. The chromosome numbers in the controls at 8 days were in the range from 41 to 87 with the modal values at 46, 47, and 48. On the other hand, in treated material the chromosome numbers were in the range from 42 to 48 with four peaks at 43, 44, 45, and 46 on the 8th day of treatment with smoke condensate. However, the chromosome numbers on the 15th, 20th, and 28th days in treated material and in controls for 30 days showed an increased spread of the range.

DESCRIPTION OF ILLUSTRATIONS

The magnification for all figures is given by the scale shown in the upper left area of the first figure in each plate, except for plate 2.

Plate 1

Photomicrographs of kitten pumonary epithelial cells, made with phase contrast microscopy, showing successive series in the course of injury to mitochondria in response to smoke condensate.

Figs. 1 to 4. 1:400 dilution.

Fig. 1. Filamentous mitochondria before treatment.

Figs. 2 to 4. The process of mitochondrial ring-formation after 23, 28 and 61 minute exposures respectively.

Figs. 5 to 8. 1:800 dilution.

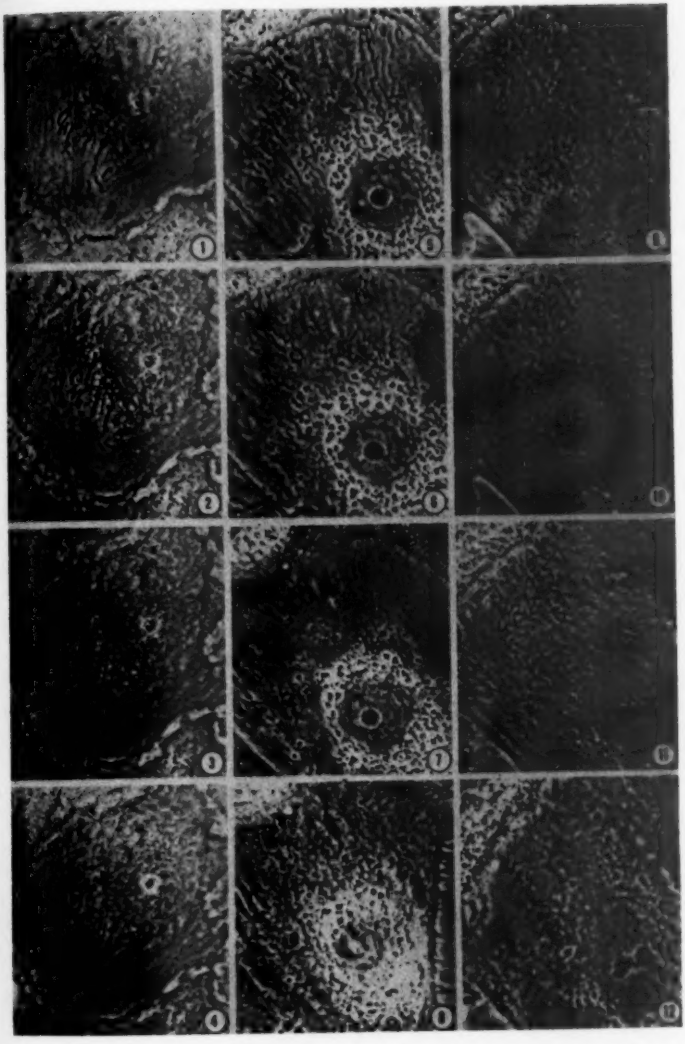
Fig. 5. Filamentous mitochondria before treatment.

Figs. 6 to 8. Fragmentation of the mitochondria with degenerative changes in the nucleus after 29, 70 and 137 minute exposures respectively.

Figs. 9 to 12. 1:1200 dilution.

Fig. 9. Filamentous mitochondria before treatment.

Figs. 10–12. Fragmentation of the mitochondria after 4, 28 and 44 minute exposures respectively.



b. *Morphological analysis of the chromosomes.* As has been noted, the most frequent chromosome numbers for the treated material on the 8th day were seen to have shifted to the left, namely, they were decreased. Some cells, in which the centromere region of the chromosomes was readily distinguishable, were selected for a critical study of the karyotype. Microscopical examination was made as accurately as possible of the individual chromosomes. They were then drawn with the aid of a camera lucida with special attention being given to their size and shape. Conspicuous changes were not observed between control and treated materials (Figs. 37–42).

II. *Development of Two Human Lung Cell Strains from Control and Treated Cultures.*

1. *Development of the strains.*

The supply of explants for both control and treated bottle cultures of a human lung was nearly exhausted for the study of chromosome numbers, and only a few mitoses could be found. On the 28th day after treatment with smoke condensate at a 1:10,000 dilution, the remaining cultures were put on Eagle's nutrient medium which was changed once a week (Figs. 43, 44).

PLATE 2

Photomicrographs of kitten pulmonary epithelial cells. Figures 13 to 16, preparations stained with Giemsa-May-Grünwald; figures 17 to 24, dark phase contrast microscopy. The magnification for figures 13 to 16 is given by the scale shown in figure 13, figures 17 to 21, 23 and 24 as shown in figure 17, and figure 22 as shown.

FIG. 13. Untreated cells.

FIGS. 14 to 16. Cells treated with smoke condensate at 1:200, 1:400 and 1:800 dilutions, showing pycnotic degeneration.

FIGS. 17 to 20. Photomicrographs made on the 2nd day in contact with smoke condensate.

FIG. 17. An untreated cell showing filamentous mitochondria.

FIG. 18. 1:600 dilution, showing rod-like mitochondria.

FIG. 19. 1:3200 dilution. Some mitochondria remained without change in form.

FIG. 20. 1:9600 dilution, showing filamentous mitochondria which closely resembled those of the untreated controls.

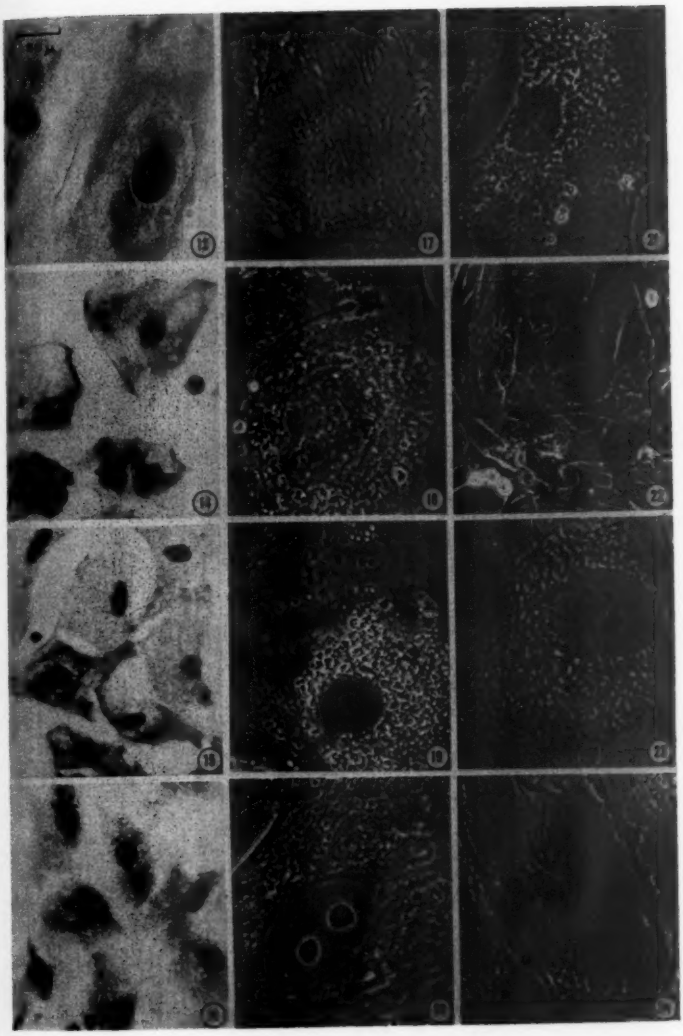
FIGS. 21 to 24. Photomicrographs made on the 7th day in contact with smoke condensate.

FIG. 21. 1:400 dilution, showing a pycnotic cell.

FIG. 22. 1:600 dilution, showing some multinucleate cells.

FIG. 23. 1:3200 dilution. The mitochondria were aggregated in a network around the nucleus.

FIG. 24. 1:6400 dilution, showing swollen mitochondria.



On the 101st day (treated) and 102nd day (control) after explantation, the first subcultures were made as follows: a rubber policeman was employed to scrape the cells from the glass. For subculture the out-growing cells were dispersed with a 30 ml. syringe fitted with a no. 15 needle, and 20 ml. of the cell suspension were put into T60 flasks, the explants being discarded. The floors of the T60 flasks were covered with a clot consisting of 0.2 ml. each of embryonic extract and heparinized cockerel plasma which was allowed to set for ten minutes before adding the cell suspension. Trypsin was not employed in preparing this suspension in order to reduce the number of chemical variables in this experiment. The cells had become attached to the glass 24 hours later and appeared to form a sheet. These elements began to multiply very rapidly on the second day. Some epithelial-like cells were observed among the fibroblast-like elements (Figs. 45, 46). On the 8th day (control) and 9th day (treated) the entire floor of the flasks were covered and second subcultures into 2 T60 flasks for each series were made without the use of trypsin (Figs. 47, 48). As shown in Figures 49 and 50, in the second subcultures some colonies of the epithelial-like elements were observed for both control and treated cells. On the 5th day after the 2nd subcultures, 467 epithelial-like cells (22.0%) out of 2118 in the control and 503 epithelial-like cells (24.2%) out of 2080 in the treated culture were observed. After the 3rd subcultures plasma clots were no longer used to cover the floors of the flasks. In the course of subculturing both the

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PLATE 3

FIGS. 25 to 30. Photomicrographs of human pulmonary fibroblast-like cells in both untreated and treated materials made with phase contrast microscopy on the 2nd day.

FIG. 25. Untreated cell showing filamentous mitochondria.

FIGS. 26 to 27. 1:400 and 1:800 dilutions, respectively, showing pycnotic cells.

FIG. 28. 1:1600 dilution showing somewhat less nuclear damage.

FIGS. 29 to 30. 1:3200 and 1:10,000 dilutions showing filamentous mitochondria.

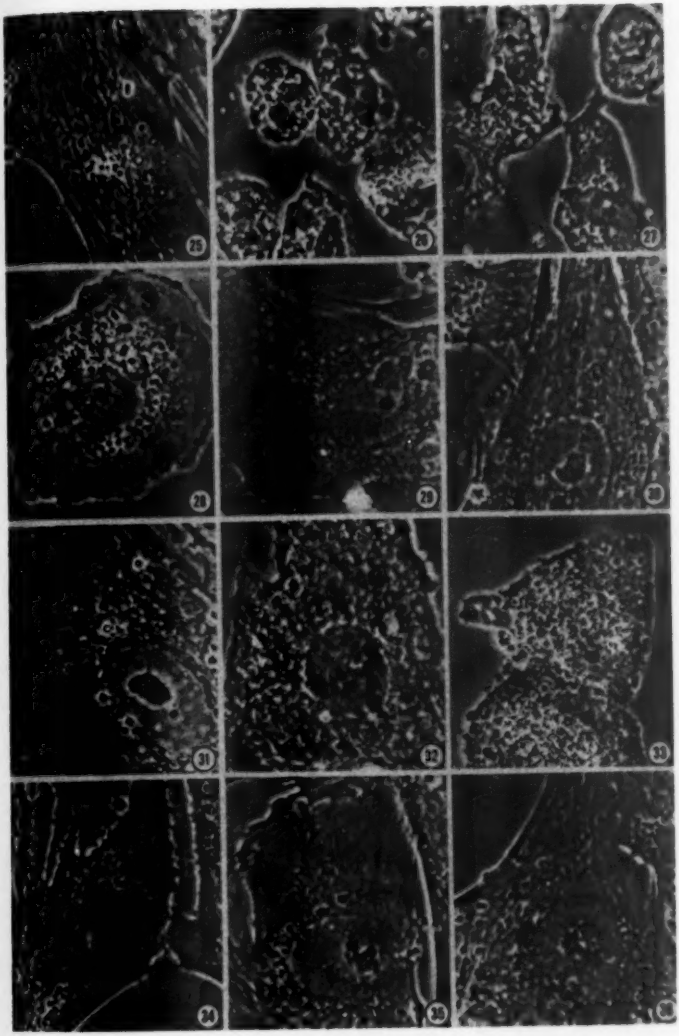
FIGS. 31 to 36. Photomicrographs of human pulmonary fibroblast-like cells in both untreated and treated material on the 7th day.

FIG. 31 Untreated cells with filamentous mitochondria.

FIGS. 32 and 33. 1:400 and 1:800 dilutions, respectively, characterized by pycnotic cells.

FIGS. 34 and 35. 1:1600 and 1:3200 dilutions showing swollen mitochondria.

FIG. 36. 1:10,000 dilution representing unchanged mitochondria.



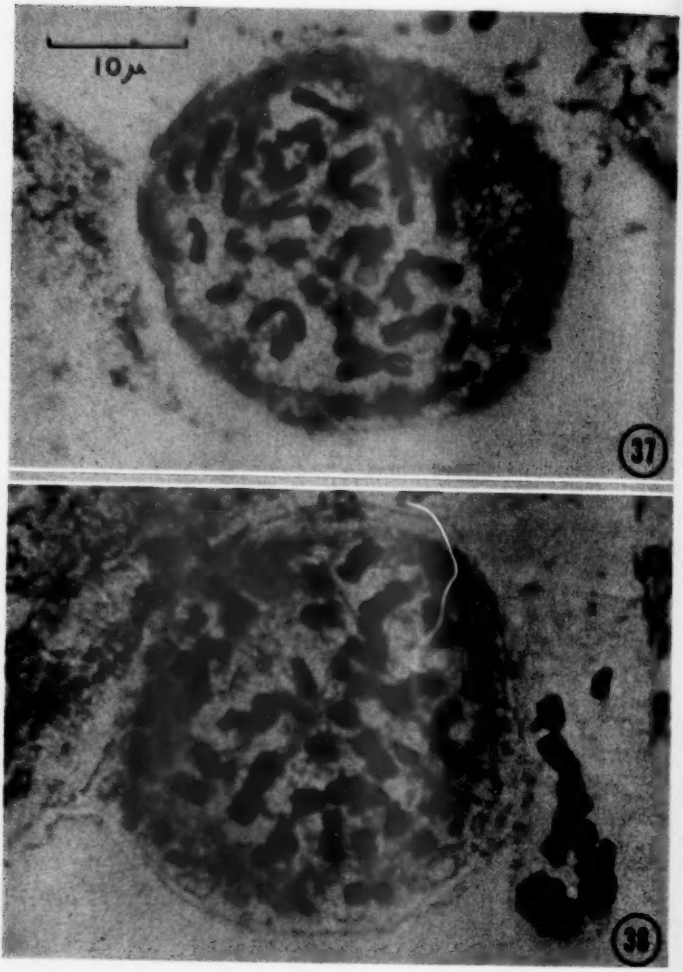


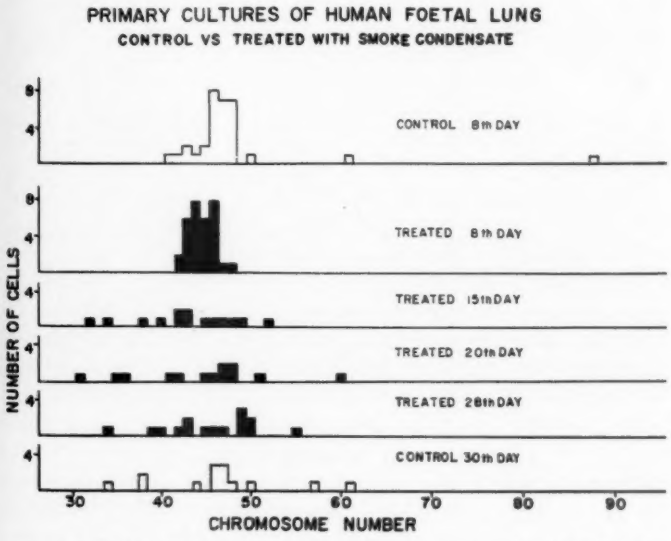
PLATE 4

FIGS. 37 and 38. Photomicrographs of the chromosomes in human lung cells. Figure 37 (control) and figure 38 (treated) correspond to the drawings shown in figures 39 and 41.

treated and control series, the number of epithelial-like cells showed a progressive dominance. In the 8th subcultures all cells appeared to be epithelial elements in both lines. Thus two human lung epithelial cell strains were established. The line derived from the control culture was designated as the HL-C strain Nakanishi, and the one from the treated culture was designated as the HL-S strain Nakanishi (Figs. 51, 52). However, since both arose at approximately the same time and subsequent chromosome analysis showed no marked difference, it is believed that pretreatment with the smoke condensate was not significantly important in the establishment of the strain. The subcultures were made every 4 to 7 days, and at the time of writing both lines were in the 107th subculture, which is about a year and 8 months from the date of the primary cultures (July 26, 1957 to March 15, 1959).

2. *Chromosomal studies of the strains.*

The chromosome numbers and karyotype of both lines were studied according to the method described by Hsu and Klatt



TEXT-FIGURE 2. Distribution of chromosome numbers in the cells of a human foetal lung. Control versus treated with smoke condensate (Ecusta).

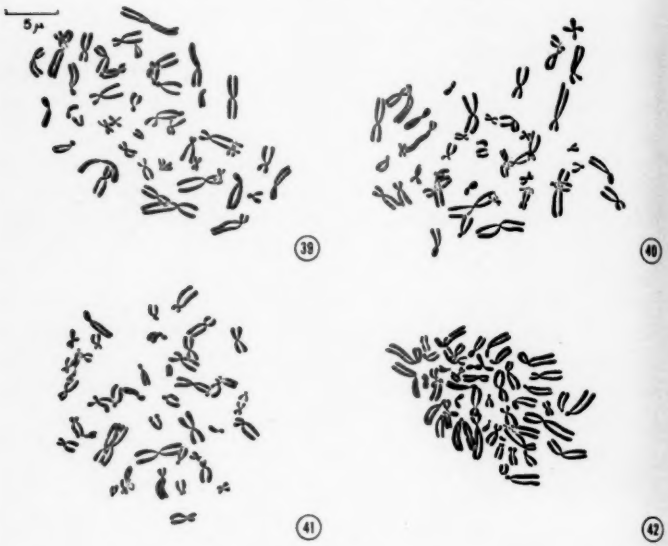


PLATE 5

Camera lucida drawings of chromosomes of human lung cells from primary culture.

FIGS. 39 and 40. Metaphase from untreated cultures with 46 and 47 chromosomes, respectively.

FIGS. 41 and 42. Metaphase from cultures treated with smoke condensate (1:10,000 dilution) for 8 and 15 days having 45 and 52 chromosomes, respectively.

(13), Nakanishi and Mizutani (24), Nakanishi (23), and Nakanishi et al (25), using the following procedure: Cultures were treated with a colchicine solution (0.4 ml. of a 1:10,000 dilution of colchicine in distilled water plus 20 ml. Eagle's medium) for 16 hours at 37° C. At the end of this period the cells were treated with a hypotonic solution (sodium citrate 1.12% in distilled water), and then they were scraped from the floors of the flasks with a rubber policeman. The cell suspension was centrifuged and the hypotonic solution decanted. It was found that the total time for hypotonic treatment, scraping, and centrifuging should be approximately 10 minutes to obtain best results. The cells in the centrifuge tube were fixed with freshly prepared Carnoy's fluid usually for 5 hours. The fixative was then replaced with

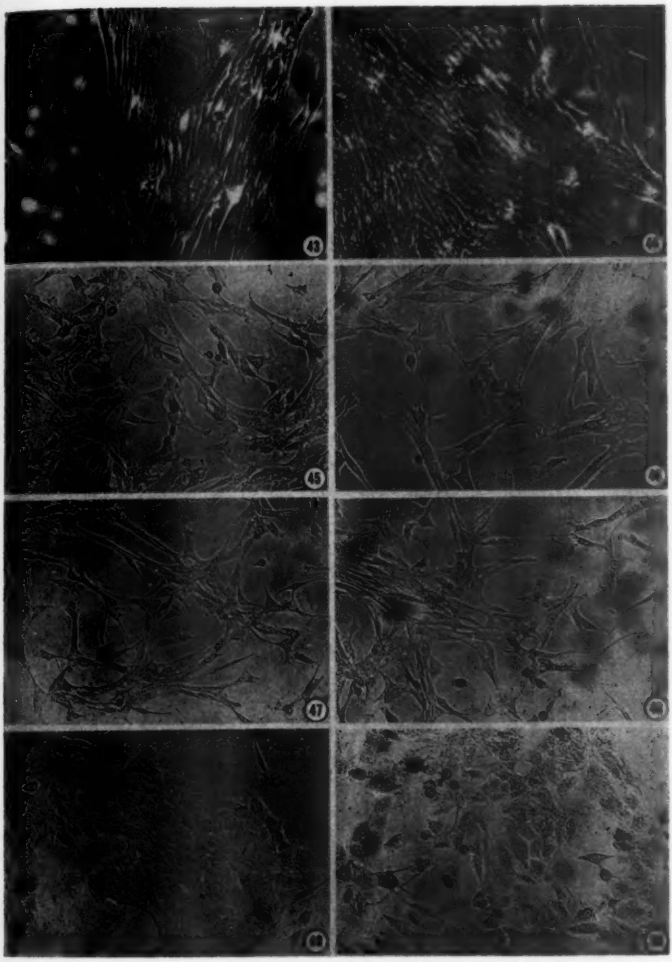
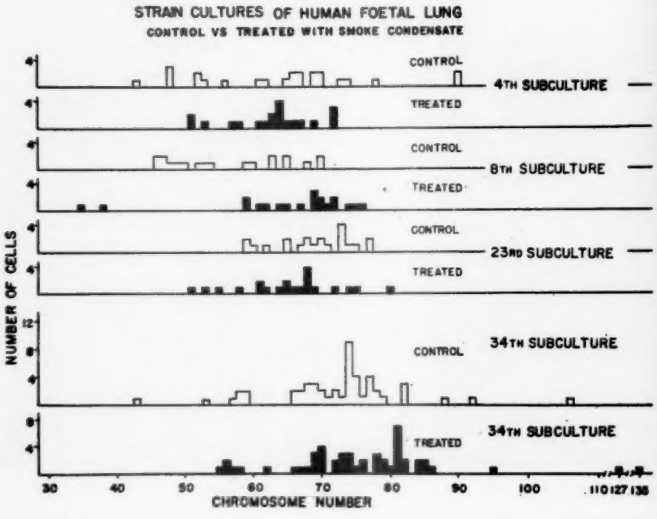


PLATE 6

Photomicrographs of human lung cells *in vitro* contrasting control and smoke condensate treated cultures.
FIGS. 43 and 44. Primary cultures.
FIGS. 45 and 46. First subcultures, showing fibroblast-like cells.
FIGS 47 to 50. Second subculures.
FIGS. 47 and 48 showing fibroblast-like cells.
FIGS. 49 and 50 showing colonies of epithelial-like cells.

approximately 0.5 ml. of 50 per cent acetic acid, and one or two drops of acetic orcein (2 grams of orcein in 100 ml. of 50% acetic acid). Small drops of the cell suspension were placed on clean glass slides and mounted with a cover slip. A piece of blotting paper was then laid over the cover slips and pressed slightly with the thumb. Finally the edges of the cover slips were sealed with Deckglaskitt nach Krönig Cement.[1] Observations were made with the use of a Zeiss phase contrast microscope (dark contrast 100x objective and a Zeiss 8x ocular) and a Bausch and Lomb phase contrast microscope (dark contrast 97x objective and a 12x ocular). Accurate chromosome counts were made from drawings of well-spread metaphase plates. Data assembled in histograms are shown in text-figure 3. In the 4th, 8th, and 23rd subcultures (134, 153, and 197 days from the date of the primary cultures, respectively) of both HL-C and HL-S strains, the chromosome numbers were distributed over a wider range with



TEXT-FIGURE 3. Distribution of chromosome numbers in the human lung cell strains derived from untreated control (HL-C) and treated cultures (HL-S).

[1] This wax can be obtained from Riedel Haën A.-G., Seelze bei Hannover, Germany.

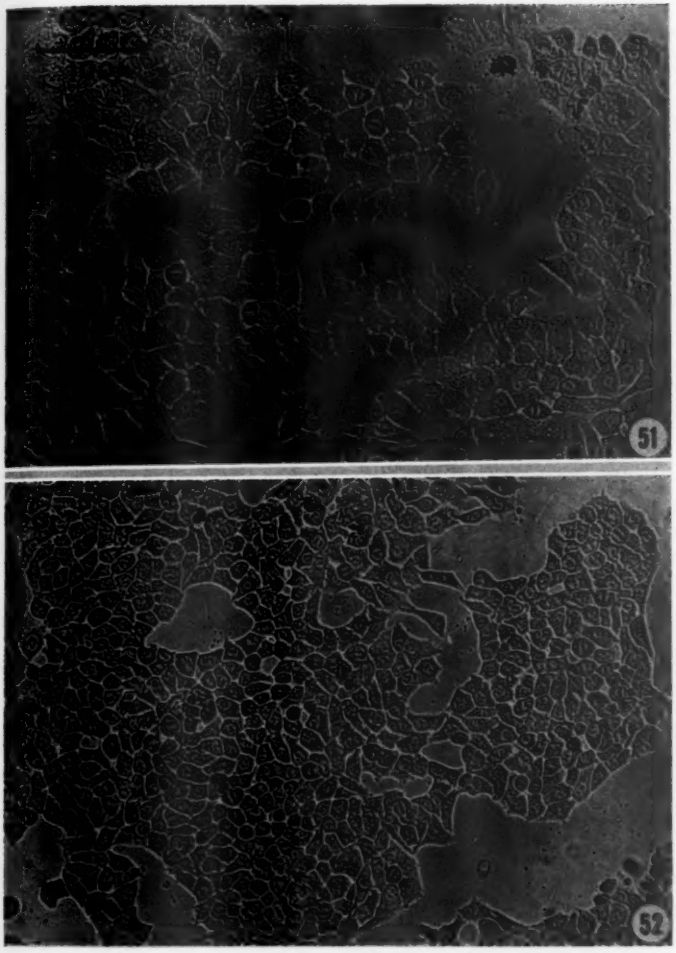


PLATE 7

FIGS. 51 and 52. Photomicrographs of human lung strains contrasting control (HL-C) and smoke condensate treated cultures for 28 days (HL-S). Thirteenth subcultures.

no high peak. In the 34th subcultures (226 days) 9 out of 50 cells in the HL-C strain had 74 chromosomes in a range from 43 to 106, and in the HL-S strain 7 out of 50 cells had 81 elements in a range from 55 to 135. Microscopical examination was made of the individual chromosomes to identify homologous pairs as accurately as possible. Each chromosome was drawn with the aid of a camera lucida with special attention being given to its size

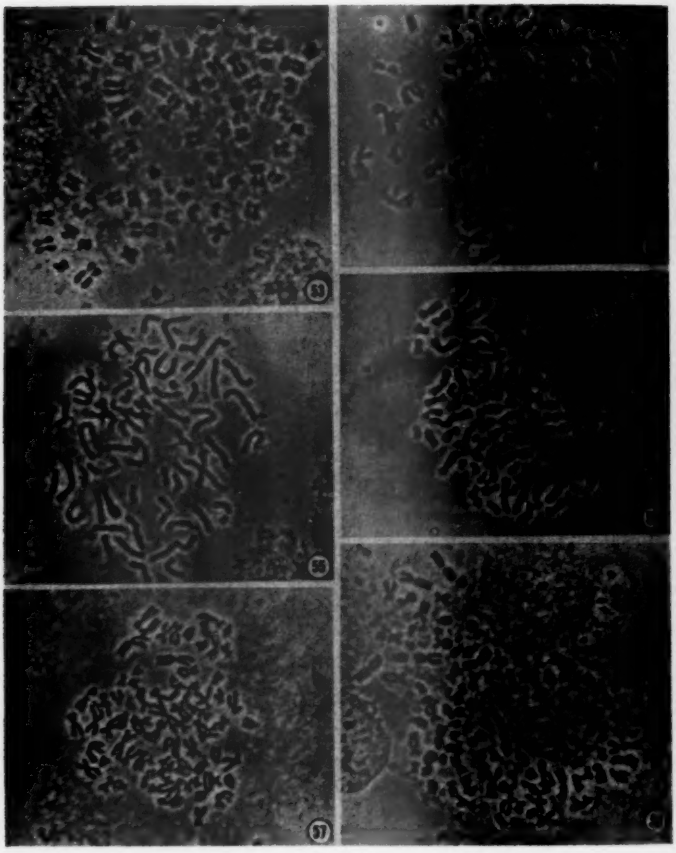


PLATE 8

FIGS. 53 to 58. Photomicrographs of the chromosomes of human lung untreated strain (HL-C).

and shape (Figs. 53–58, 59–63, 64–67). The drawings were then photographed, and from prints individual chromosomes were cut out with the aid of a pair of scissors. These were placed in serial alignment according to size and the locus of the centromeres. Figures 68–71 show such ideograms of chromosomes of the HL-C and HL-S strains.

On the 353rd day (61st subculture) 30 metaphase figures of the HL-C strain were observed. The chromosome numbers were in a narrower range from 65 to 96 with a reasonably high peak at 77 (43%). Further, in the counts of 50 metaphase plates of the HL-S strain on the 397th day (71st subculture), the most frequent chromosome number was 76 (26%) in a range from 69 to 80 (text-fig. 4).

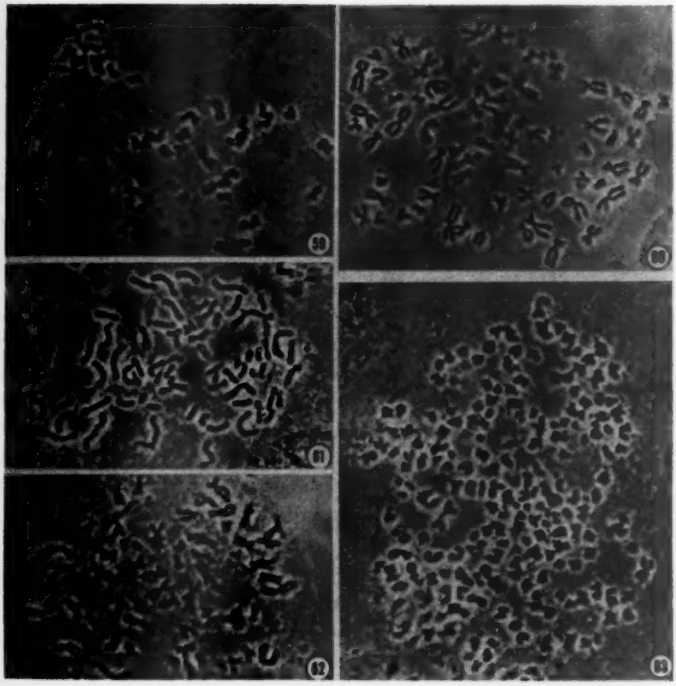
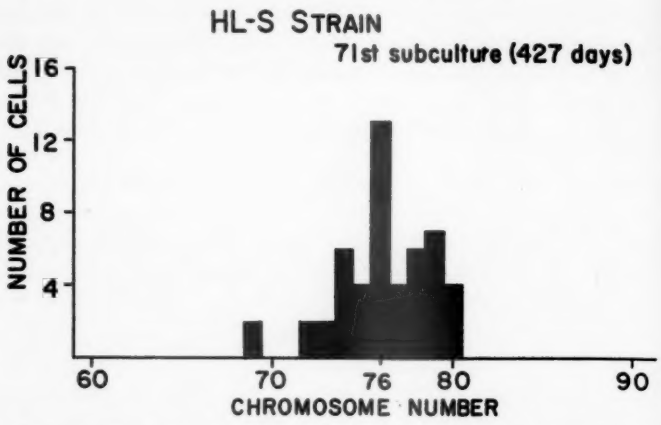


PLATE 9

FIGS. 59 to 63. Photomicrographs of the chromosomes of human lung smoke condensate treated strain (HL-S).



TEXT-FIGURE 4. Distribution of chromosome numbers in the human lung cell strain derived from the culture treated with smoke condensate (HL-S strain).

III. *Chromosomal Studies on the Effect of Neal's Smoke Condensate on the Cells of a Human Lung Strain (HL-C Nakanishi).*

In contrast to primary cultures, cells of strain lines have a higher mitotic rate, their modal chromosomal number is relatively fixed, and they lend themselves more readily for quantitative studies. It was on the basis of these considerations that the strain of human pulmonary epithelium (HL-C) was employed to recheck chromosomal injury which might result from long-term contact with fractions of smoke condensates.

As described in the previous section, the 61st subculture of the HL-C strain yielded a modal value of 77 chromosomes. It was considered that this number would remain constant in future subcultures. This assumption proved to be correct.

As a preliminary experiment to determine the suitable dosage at which the cells could continue to multiply in contact with a chronic application of smoke condensate, T30 flask cultures of the HL-C strain were treated with Neal's No. 1 and No. 3 fractions at concentrations of 0.01 mg. and 0.1 mg. per ml. in Eagle's medium together with corresponding acetone (0.002 ml. of acetone per ml. in Eagle's medium) and untreated controls. It was

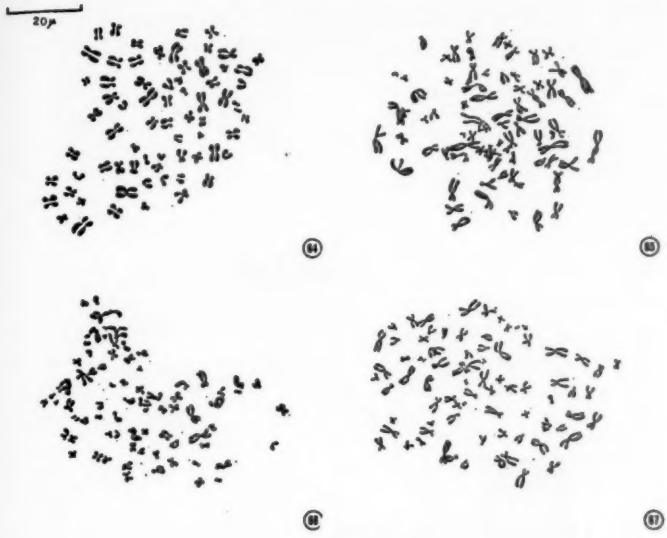


PLATE 10

Camera lucida drawings of chromosomes of human lung strains.
FIGS. 64 and 65. HL-C strain, 69 and 77 chromosomes, respectively.
FIGS. 66 and 67. HL-S strain, 78 chromosomes, respectively.

necessary to prepare the acetone control, because the fractions were dissolved in this solvent as an intermediate agent for mixture with Eagle's medium.

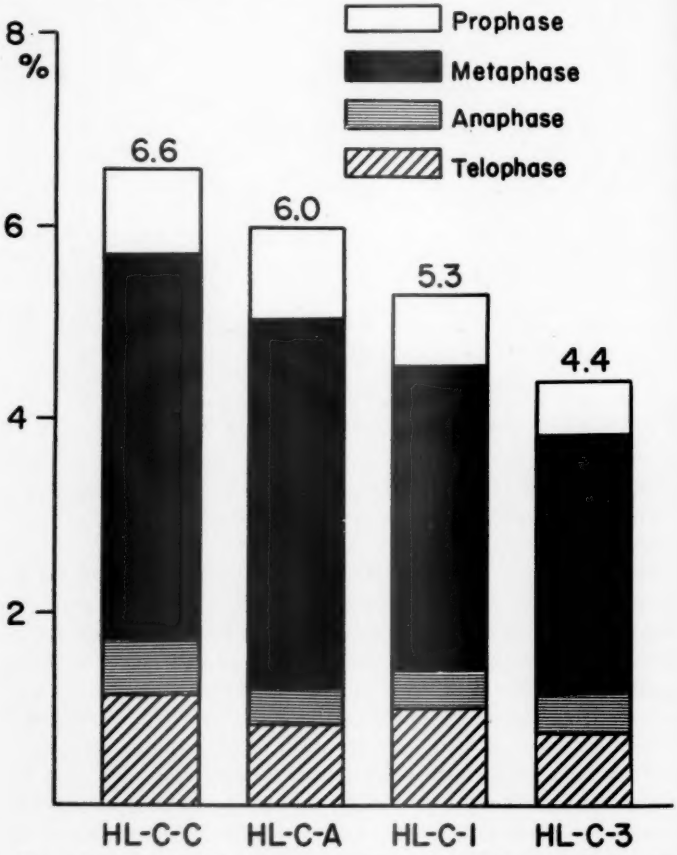
On the 6th day after treatment photomicrographs were made through the floor of the culture flasks (Figs. 72–77). For the untreated and acetone controls as well as for the series containing 0.01 mg. of the No. 1 and No. 3 fractions, average mitoses were observed, and the cells seemed to be healthy. However, in the cultures treated with 0.1 mg. of the No. 1 and No. 3 fractions very few mitoses were found, especially in the No. 3 series where most of the cells had been killed by this time.

On the basis of these results, it was decided to set up 4 experimental conditions with the use of the following symbols: HL-C-C, untreated control; HL-C-A, acetone control (0.002 ml. of acetone per ml. of Eagle's medium); HL-C-1 and HL-C-3, treated with 0.01 mg. of the No. 1 and No. 3 fractions per ml. of Eagle's

medium, respectively. Under these experimental conditions many mitotic cells were observed (text-fig. 5).

1. *Chromosome counts.*

Employing the hemocytometer counting technique, approximately 70,000 cells per ml. of Eagle's medium were dispensed in T60 flasks of each category (20 ml. per flask). The cultures were



TEXT-FIGURE 5. Mitotic index of the HL-C strain cells treated with #1 and #3 fractions of Neal's smoke condensate (HL-C-1 and HL-C-3 series) together with corresponding acetone and untreated controls (HL-C-A and HL-C-C).

treated with acetone and with the No. 1 and No. 3 fractions of the smoke condensate, as shown in text-figure 6, respectively. At each passage the chromosome counts were made of smoke condensate-treated cells together with corresponding acetone and untreated controls in the 1st, 2nd, 4th, 6th, 7th, 8th, 9th, 10th, 14th, 15th, 18th, and 20th cultures (5, 9, 15, 25, 30, 35, 40, 45, 65, 70, 85, and 95 days after treatment, respectively). For this experiment a total of 1080 metaphase figures with well-spread chromosomes were carefully drawn and counted. Results are presented in tables 1–4 and text-figure 7. In the course of 20 passages (95 days) the most frequent chromosome number for the control cultures was constantly at 77. In contrast the values for the treated cells were scattered over a wide range on the 5th, 8th, and 15th days. However, on the 25th day the most frequent chromosome number was 77. On the 35th day the modal value

PLATE 11

Ideograms for the cells of the human lung strains (HL-C and HL-S).
FIGS. 68 and 69. HL-C strain corresponding to figures 53 and 64, and figures 54 and 65, respectively.
FIGS. 70 and 71. HL-S strain, corresponding to figures 59 and 66, and 60 and 67, respectively.

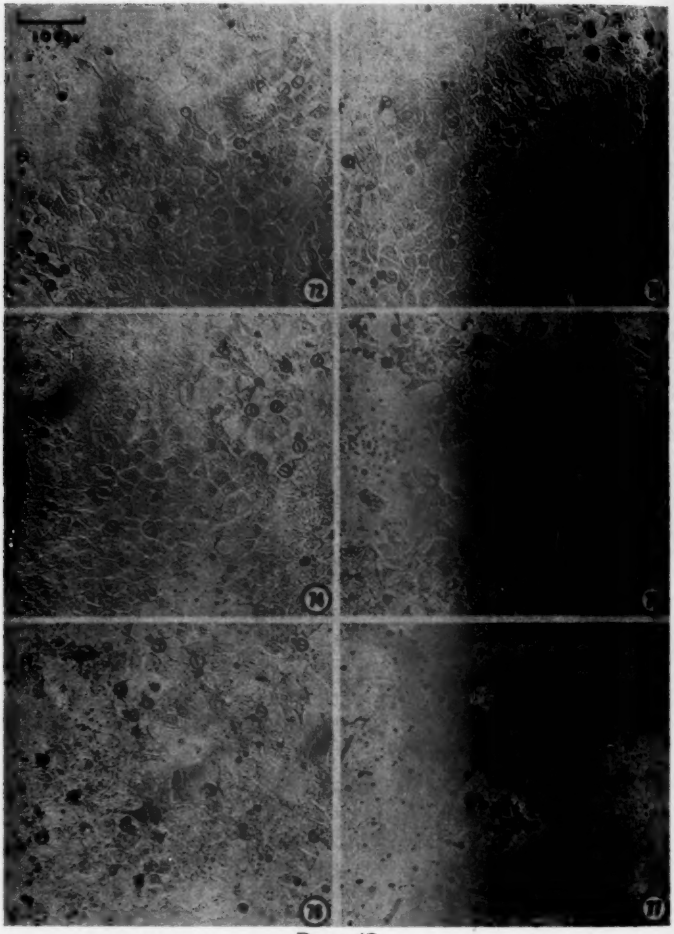


PLATE 12

Photomicrographs made on the 6th day of the cells of the HL-C strain treated with the #1 and #3 fractions of Neal's smoke condensate at concentrations of 0.01 mg. and 0.1 mg. per ml. in Eagle's medium together with corresponding acetone and untreated controls.

FIG. 72. Untreated cells.

FIG. 73. Acetone control cells.

FIGS. 74 and 75. Cells treated with the #1 fraction, 0.01 mg. and 0.1 mg., respectively.

FIGS. 76 and 77. Cells treated with the #3 fraction, 0.01 mg. and 0.1 mg., respectively.

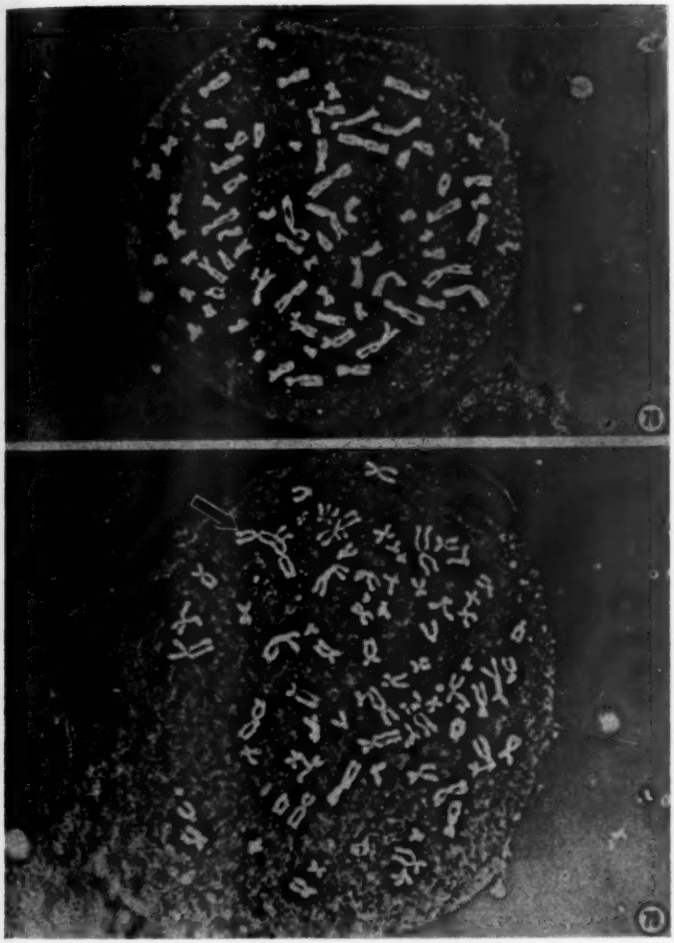


PLATE 13

Photomicrographs of the chromosomes of the HL-C strain cells treated with the #3 fraction of Neal's smoke condensate together with untreated control culture.

FIG. 78. Untreated control cell (HL-C-C series).

FIG. 79. Treated cell (HL-C-3 series in the 7th passage. The arrow indicates the dicentric chromosome.

fell to 76, and this persisted until the latest observation on the 95th day.

As a further check on the effect of smoke condensate, a higher dosage than that of the previous experiment was employed. A T60 flask culture of the HL-C strain was treated with the No. 3 fraction at a dosage of 0.1 mg. per ml. of Eagle's medium. As has

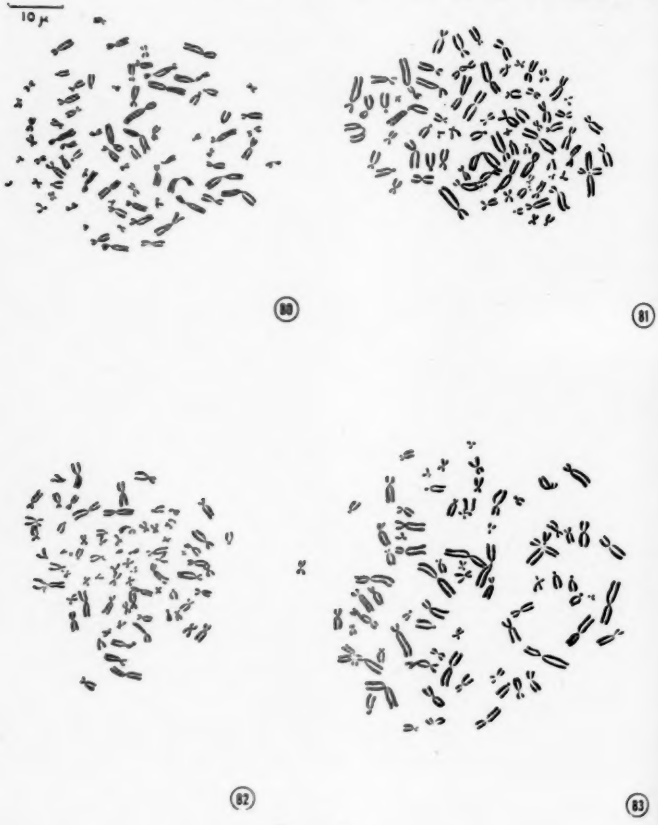
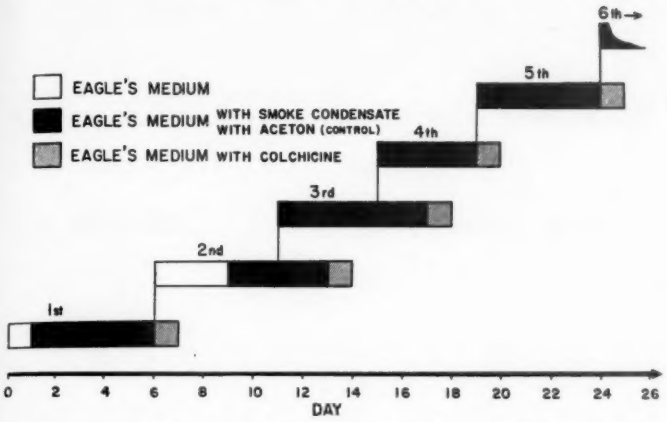


PLATE 14

Camera lucida drawings of the chromosomes with the modal value of 77 in the untreated cultures (HL-C-C) and elements with the new modal value of 76 in the treated culture (HL-C-3).
FIGS. 80 to 82. 77 chromosomes, respectively.
FIG. 83. 76 chromosomes.

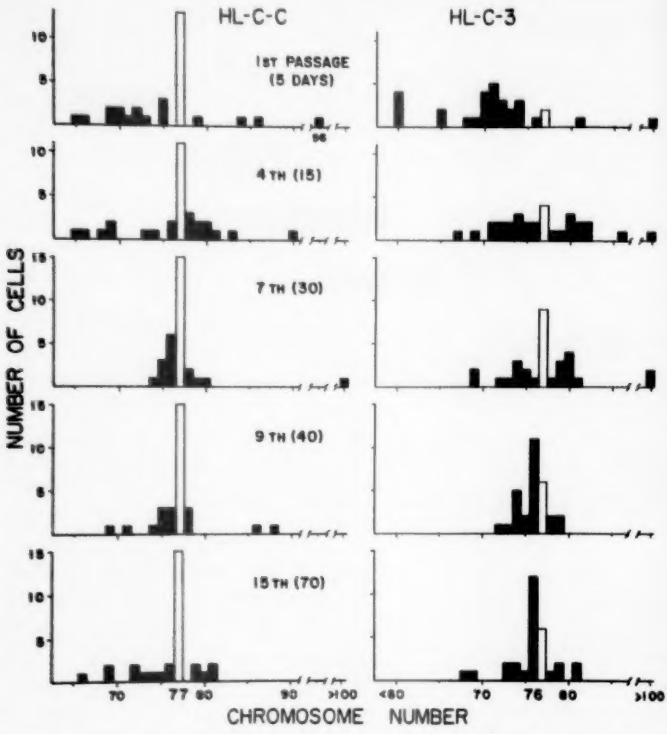


TEXT-FIGURE 6. Procedure for treating the cells of the HL-C strain with the #1 and #3 fractions of Neal's smoke condensate at a concentration of 0.01 mg. per ml. in Eagle's medium together with corresponding acetone control (0.002 ml. of acetone per ml. in Eagle's medium).

been described smoke condensate at this dosage killed the most cells within 6 days (Fig. 77). Therefore, at each passage exposure was limited to 8 to 29 hours, depending upon the appearance of the cells (text-fig. 8). Results are presented in table 5 and text-figure 9. In the first culture of this series the chromosome numbers showed two peaks at 76 (7 out of 30 cells) and 77 (9 out of 30 cells) in a range of 64 to 157. In the 2nd passage the most frequent chromosome numbers shifted to 76 which was identical with that observed in a series of HL-C-3 (0.01 mg. of No. 3 fraction per ml.) in a range of 72 to 158. However, in the 3rd passage the chromosome number again showed two peaks of the same height at 76 and 77 (8 out of 30 cells, respectively) in a range of 69 to 79. Unfortunately this series of experiments was accidentally lost.

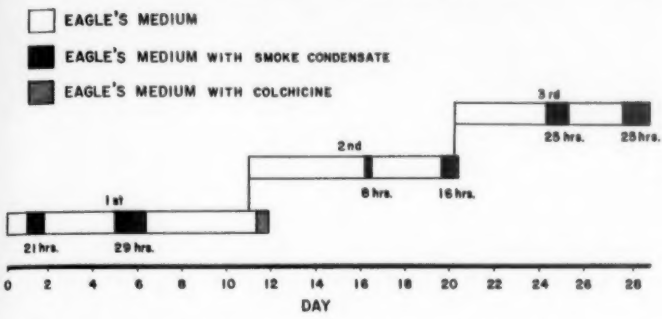
2. *Development of dicentric chromosomes.*

In the period during which the change of the chromosome numbers occurred, dicentric chromosomes appeared (Fig. 79) in the 7th and 8th passages both of HL-C-1 and HL-C-3 and in each subsequent passage of the 0.1 mg. of No. 3 fraction series (Table 6) in comparison with the control (Fig. 78). However, dicentrics



TEXT-FIGURE 7. Distribution of chromosome numbers in cells treated with the #3 fraction (HL-C-3 series) together with untreated control (HL-C-C series). Representative passages were selected from Table 1 and 4.

no longer appeared after the 8th passage of both HL-C-1 and HL-C-3 lines. In the material receiving colchicine-hypotonic pretreatment, the only locus that held the sister chromatids together was at the centromere, and therefore both constrictions of the dicentric apparently were centromeric and not secondary indentations. All cells with dicentric chromosomes were drawn with the aid of a camera lucida. Figures 80–87 illustrate representative cells from this series. The lengths of each part of the dicentric chromosome, using the symbols of (a) for the shortest arm, (b) for the segment between two centromeres, and (c) for the remaining arm, were measured on the basis of 14 metaphase plates



TEXT-FIGURE 8. Procedure for treating cells with the #3 fraction of Neal's smoke condensate at a concentration of 0.1 mg. per ml. in Eagle's medium.

drawn at the same magnification with the aid of a flexible millimeter rule. The measurements were converted into microns, and the results are presented in Table 7. The dicentric chromosomes were roughly classified into four groups as follows: a=b=c (#1, #4, and #9 dicentric sets), a=c<b (#5, #6, #10, #11, and #13), a<b<c (#2, #7, and #12), and a<c<b (#3, #8 and #14). The average ratio of a, b, and c was 1:2.3:1.4.

In order to analyze the possible mechanism of the formation of the dicentric chromosomes, three ideograms from the cells with the modal value of 77 in the HL-C-C and one with the new modal value of 76 in the HL-C-3 series, as well as three ideograms from the cells with 75 chromosomes, plus one dicentric element in the HL-C-1 and HL-C-3 series, are presented in Figures 88–94. The serial alignments of 77 chromosomes of the HL-C-C indicated that there were 37 pairs which were homologous and 3 rather small nonhomologous elements. The first three pairs were V-shaped with metacentric centromeres, the fourth pair was J-shaped with a submetacentric centromere, and other pairs were with V- or J-shaped elements (Figs. 88–90). In contrast, in the ideograms of the dicentric chromosome sets (Figs. 92–94), there were 36 pairs of homologous chromosomes or one pair less than those in the HL-C-C, 3 non-homologous elements and a dicentric. In each serial alignment of the dicentric chromosome sets, out of 3 non-homologous chromosomes two were longer than those in the HL-C-C, especially one of them

TABLE 1

Distribution of chromosome numbers in the cells of untreated controls (HL-C-C) in relation to culture passage. Based on 30 metaphase figures for each passage in this and Tables 2, 3, 4, 5, 8 and 9

Passage Number	Chromosome Number																								
	60	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	83	84	87	89	90	91	96	>100
1 (5)*	..	1	1	..	..	2	2	1	2	1	..	3	..	13	..	1	1	..	..	..	1	..	..	..	1	..
2 (9)	1	..	..	1	2	..	2	2	2	..	2	1	1	7	2	2	1	2	1	1	..	..	..	..	..	..
4 (15)	..	1	1	..	1	2	..	..	..	1	1	..	2	11	3	2	2	1	1	..	..	..	1	..	..	..
6 (25)	..	..	..	2	..	..	..	1	1	3	3	1	4	11	1	1	1	1	..	..	..	..	..	..	..	..
7 (30)	..	..	..	..	..	..	..	..	..	..	1	3	6	15	2	1	1	..	..	..	..	..	..	..	..	1
9 (40)	..	..	..	..	..	1	..	1	..	..	1	3	3	16	3	..	..	..	..	..	1	1	..	..	..	..
15 (70)	..	..	1	..	..	2	..	..	2	1	1	1	2	15	..	2	1	2	..	..	..	..	..	..	..	..
18 (85)	..	..	..	..	..	1	..	..	1	..	2	4	6	13	2	1	..	..	..	..	..	..	..	..	..	..
20 (95)	..	..	..	..	..	..	1	..	..	..	3	6	5	12	..	1	..	1	..	..	..	..	..	1	..	..
26 (125)	..	..	1	..	..	..	2	1	..	..	1	4	5	13	..	2	..	..	1	..	..	..	..	..	..	..

* Lapse of time in days.

TABLE 2

Distribution of chromosome numbers in the cells of the acetone control (HL-C-A)in relation to culture passage

Passage Number	Chromosome Number																					
	45	49	61	63	66	67	69	70	71	72	73	74	75	76	77	78	79	80	81	82	85	90
1 (5)*	1	..	1	..	..	..	..	..	1	1	3	4	4	4	8	3	..	..	..	..	..	..
2 (9)	..	..	..	1	1	..	1	1	2	4	..	1	3	2	7	1	1	2	2	1	..	..
4 (15)	..	..	..	..	..	1	..	..	..	..	2	4	2	2	11	3	1	1	2	..	1	..
6 (25)	..	1	..	..	..	..	1	2	1	2	2	4	1	1	11	1	1	..	1	..	..	1
7 (30)	..	..	..	..	..	..	..	1	..	2	1	3	2	3	15	1	1	1	..	..	..	..
9 (40)	..	..	..	..	..	..	2	..	..	..	1	3	4	3	12	..	3	2	..	..	..	..

* Lapse of time in days.

TABLE 3

Distribution of chromosome numbers in the cells treated with Neal's #1 fraction of smoke condensate (HL-C-1) in relation to culture passage.

3 (20)	..	..	..	..	..	..	..	..	..	2	..	..	..	1	3	4	3	12	..	3	2	..	..	..	..	..	..	..	..	..	..	..	..

* Lapse of time in days.

TABLE 3

Distribution of chromosome numbers in the cells treated with Neal's #1 fraction of smoke condensate (HL-C-1) in relation to culture passage.

Passage Number	Chromosome Number																																
	42	45	49	50	57	62	63	64	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	84	88	91	93	94	>100
1 (5)*	1	1	2	2	2	1	1	1	..	1	3	1	..	1	1	2	..	..	3	5	1	..	..	..	..	..	..	..	..	..	1
2 (9)	..	..	1	..	..	..	..	..	..	..	..	2	..	1	..	..	..	2	2	7	4	3	4	1	1	1	1	..	..	..	..
4 (15)	..	..	..	..	..	..	..	1	..	..	..	1	..	1	1	3	5	..	2	7	4	..	2	1	1	..	..	..	1	..	..
6 (25)	..	..	..	..	..	..	..	..	..	..	1	2	..	1	2	1	1	1	4	14	2	1	..	..	..	..	..	..	..	..	..
7 (30)	..	..	..	..	..	..	..	..	..	1	..	..	1	1	..	..	4	1	6	12	..	2	..	..	..	..	..	..	..	..	2
8 (35)	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	2	4	9	12	3	..	..	..	..	..	..	..	..	..	..
9 (40)	..	..	..	..	..	..	..	..	..	..	..	2	..	..	..	..	2	2	10	10	..	3	..	..	..	..	..	..	..	1	..
10 (45)	..	..	..	..	..	..	..	..	1	..	..	1	..	..	..	2	3	1	11	7	..	3	..	..	..	..	..	1	..	..	..
14 (65)	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	4	..	11	8	1	3	..	1	..	..	..	..	..	..	1

* Lapse of time in days.

TABLE 4

Distribution of chromosome numbers in the cells treated with Neal's #3 fraction of smoke condensate (HL-C-3) in relation to culture passage

Passage Number	Chromosome Number																														
	47	52	55	60	62	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	86	98	>100
1 (5)*	1	1	1	1	..	..	2	..	..	1	1	4	5	3	2	3	..	1	2	..	..	..	1	..	..	..	..	1
2 (9)	..	..	1	..	1	1	..	..	1	..	..	1	2	3	3	4	1	3	3	2	1	1	..	1	..	..	..	1
4 (15)	..	..	..	..	..	..	..	..	1	..	1	..	2	2	2	3	2	2	4	1	1	3	2	2	..	1	..	1
6 (25)	..	..	..	..	..	..	..	1	..	1	..	..	..	1	1	3	3	2	9	5	2	..	..	1	..	..	1	..
7 (30)	..	..	..	..	..	..	..	..	..	..	2	..	..	1	1	3	2	1	9	1	3	4	1	..	..	..	..	2
8 (35)	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	1	2	2	8	10	1	2	1	1	..	..	..	1
9 (40)	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	1	5	2	11	6	2	2	..	..	..	..	..	..
10 (45)	..	..	..	..	..	..	..	..	..	..	1	..	..	1	1	2	1	13	5	1	3	1	..	..	1	..	..	..
14 (65)	..	..	..	..	..	..	..	..	..	..	..	2	2	1	1	3	2	14	5	..	..	..	..	..	..	..	..	..
15 (70)	..	..	..	..	..	..	..	..	..	1	1	..	..	..	2	2	1	12	6	1	2	..	2	..	..	..	..	..
18 (85)	..	..	..	..	..	..	..	..	1	..	1	..	..	..	..	..	1	2	13	9	1	1	1	..	..	..	..	..
20 (95)	..	..	..	..	..	..	..	..	..	1	..	..	2	1	..	3	4	9	7	..	2	..	1	..	..	..	..	..

* Lapse of time in days.

TABLE 5

Distribution of chromosome numbers in the cells treated with Neal's #3 fraction (0.1 mg. per ml.) in relation to culture passage

Passage Number	Chromosome Number												
	64	69	72	73	74	75	76	77	78	79	81	82	>100
1	1	1	1	..	3	2	7	9	..	1	1	2	2
2	..	..	1	..	4	1	13	6	1	2	..	..	2
3	..	2	..	1	4	2	8	8	3	2	..	..	..

TABLE 6

Number of cells with dicentric chromosomes

	Passage (days)	Chromosome number									Percent
		56	64	72	74	75	76	77	>100		
HL-C-1	7th (30)	..	..	..	..	..	..	..	1	1	6.6
	8th (35)	..	..	..	..	..	..	1	..	..	3.3
HL-C-3	7th (30)	1**	..	..	..	..	..	..	..	..	3.3
	8th (35)	..	..	..	..	..	1	2	..	1	13.2
HL-C-3 (0.1 mg.)	1st (50 hrs.)*	..	..	..	..	..	..	1	..	..	3.3
	2nd (74 hrs.)*	..	..	..	1	..	..	..	..	..	3.3
	3rd (124 hrs.)*	..	..	..	..	..	1	..	..	..	3.3
HL-C-B	4th (22)	..	..	..	..	..	1	1	..	..	6.6
	5th (29)	..	..	..	..	1	..	..	..	..	3.3
HL-C-B-3	2nd (15)	..	1	1	..	1	..	..	..	..	9.9
	3rd (22)	..	..	..	..	..	..	1	..	..	..
Total		1**	1	1	1	2	3	6	1	2	..

* The time that cells were exposed to Neal's #3 fraction of smoke condensate.
** Represented by chromosome fragments not clearly associated with a particular cell.

which was the longest element among all the chromosomes except the dicentric. Therefore, it was established that one long homologous chromosome of the first pair and another fairly large-sized one were absent (black dots), as compared with the ideagrams of the HL-C-C. The remaining non-homologous chromosome was approximately the same size as those of the HL-C-C series.

From the data shown in the ideograms it seemed probable that a dicentric chromosome was formed by the fusion of two non-homologous chromosomes. Text-figure 10 illustrates this hypoth-

esis diagrammatically; namely, that one end of each chromosome was cut off, and these small fragments without centromeres might be lost. At the broken ends of the remaining elements with centromeres, fusing affinity might be increased. Finally, the fusion of the two remaining chromosomes formed a dicentric element.

The ideogram obtained from the cell with a new modal value of 76 chromosomes in the HL-C-3 indicated that there were 36 pairs of homologous chromosomes and 4 non-homologous ele-

TABLE 7

Measurement of dicentric chromosomes (microns)
a—shortest arm; b—segment between centromeres; c—the remaining arm

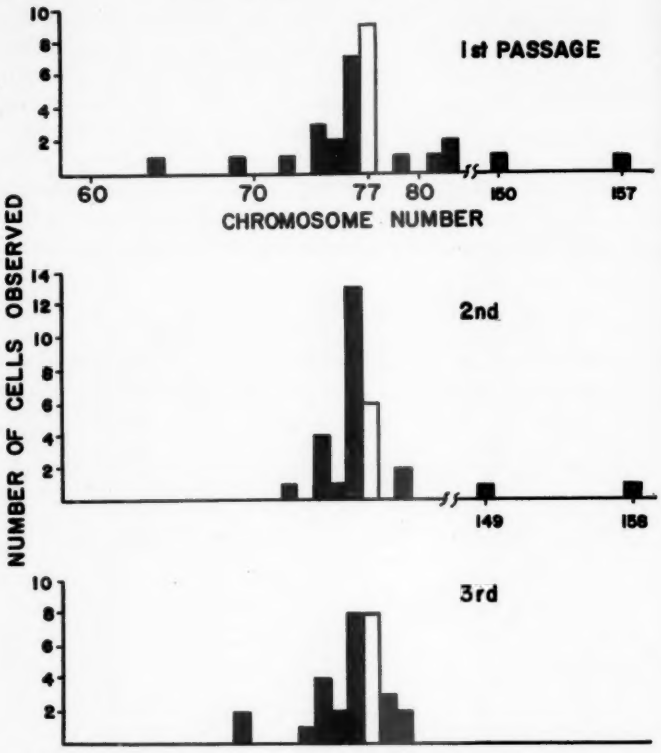
No.	a	b	c	Total	Ratio of a:b:c
1	3.6	5.4	3.3	12.3	1:1.5:0.9
2	2.0	3.1	3.8	8.9	1:1.6:2.0
3	2.0	5.6	5.1	12.7	1:2.8:2.5
4	2.8	3.1	3.1	9.0	1:1.1:1.1
5	2.7	9.0	3.3	15.0	1:3.3:1.2
6	2.7	9.8	3.1	15.6	1:2.3:0.7
7	2.1	3.3	4.3	9.7	1:1.5:2.0
8	1.2	3.7	2.7	7.6	1:3.1:2.3
9	2.5	1.8	2.5	6.8	1:0.7:1.0
10	2.1	4.1	2.1	8.3	1:2.0:1.0
11	1.1	3.1	1.2	5.4	1:2.9:1.1
12	1.0	3.5	4.0	8.5	1:3.5:4.0
13	4.1	8.2	3.9	16.2	1:2.0:1.0
14	1.6	7.4	3.7	12.7	1:4.5:2.2
Avgs.	2.3	5.1	3.3	10.6	1:2.3:1.4

TABLE 8

Distribution of chromosome numbers in the cells treated with benzpyrene alone (HL-C-B) in relation to culture passage

Passage Number	Chromosome Number													
	68	69	70	71	72	73	74	75	76	77	78	79	80	>100
1 (7)*	..	..	1	..	..	..	3	4	3	13	1	3	2	..
2 (15)	1	..	1	..	2	3	4	2	2	9	3	2	..	1
4 (22)	..	..	2	1	..	..	4	7	7	8	..	1	..	..
5 (29)	..	1	..	..	..	1	5	3	10	5	1	4	..	..

* Lapse of time in days.



TEXT-FIGURE 9. Distribution of chromosome numbers in the HL-C strain cells treated with the #3 fraction at a concentration of 0.1 mg. per ml. in Eagle's medium.

ments (Fig. 91). One of the non-homologous chromosomes was an especially long submetacentric element having a length lying approximately between that of the 7th and 9th elements.

IV. *Chromosomal Studies on the Effect of Benzpyrene Alone and in Combination with a Smoke Condensate.*

As has been described in the previous section, smoke condensates in the medium used for the cultivation of a human lung epithelial cell strain produced shifts in the modal value and the development of dicentric chromosomes. Gellhorn (10) recently

reported that the rate of development of carcinoma in mice with papillomas receiving benzpyrene plus a tobacco tar was significantly greater than in mice treated with benzpyrene alone or with the tobacco tar alone. In order to check the possibility of cocarcinogenic action, T60 flask cultures of strain HL-C were

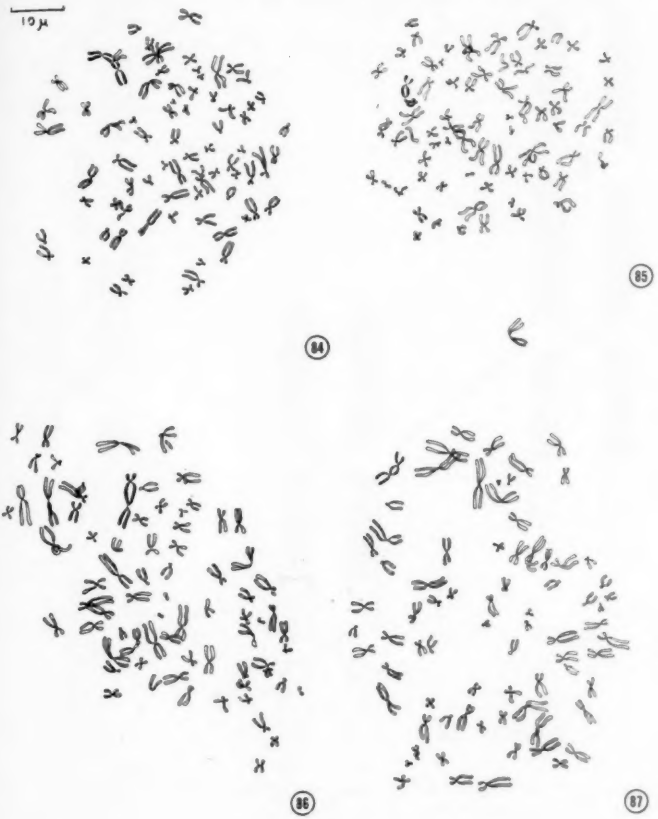
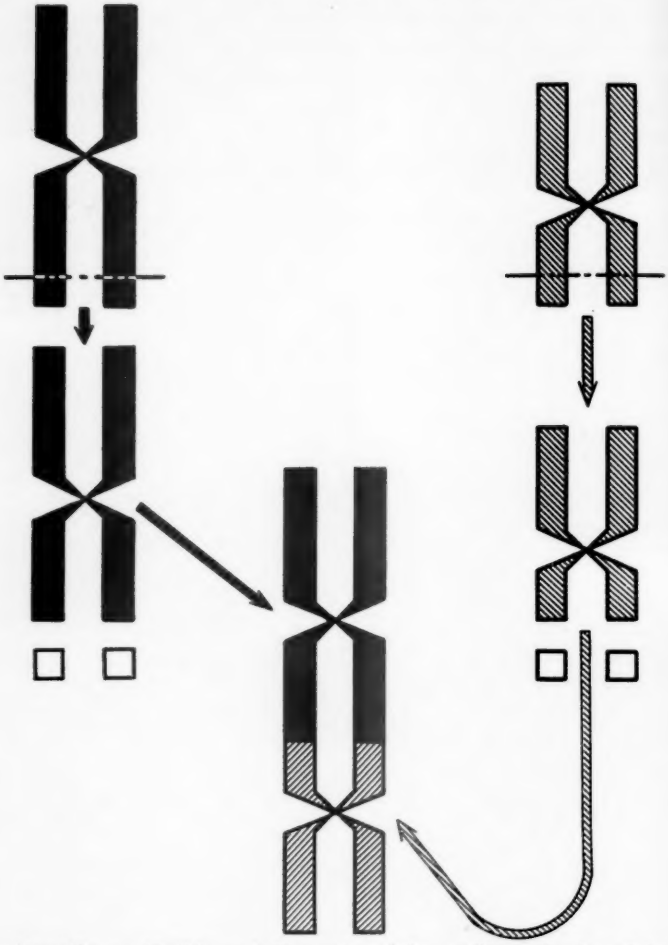


PLATE 15

Camera lucida drawings of the dicentric chromosome sets in the smoke condensate treated cultures (HL-C-1 and HL-C-3 series).
FIGS. 84 and 85. 76 chromosomes in the 8th passage of the HL-C-3).
FIG. 86. 76 chromosomes in the cell treated with the #3 fraction (0.1 mg. per ml.).
FIG. 87. 77 chromosomes in the 7th passage of the HL-C-1.



TEXT-FIGURE 10. Schematic illustration of the possible mechanism of the dicentric chromosome formation.

treated with benzpyrene alone (HL-C-B) and in combination with the No. 3 fraction of Neal's smoke condensate (HL-C-B-3).

For this experiment a total of 300 metaphase figures with well-spread chromosomes was carefully drawn and counted. Results are presented in tables 8 and 9 and text-figure 11. During

PLATE 16

Ideograms of the chromosomes with the modal value of 77 in the untreated culture (HL-C-C) and elements with the new modal value of 76 in the treated cultures (HL-C-1 and HL-C-3).
FIGS. 88 to 90. Corresponding to figures 80 to 82, respectively.
FIG. 91. Corresponding to figure 83.

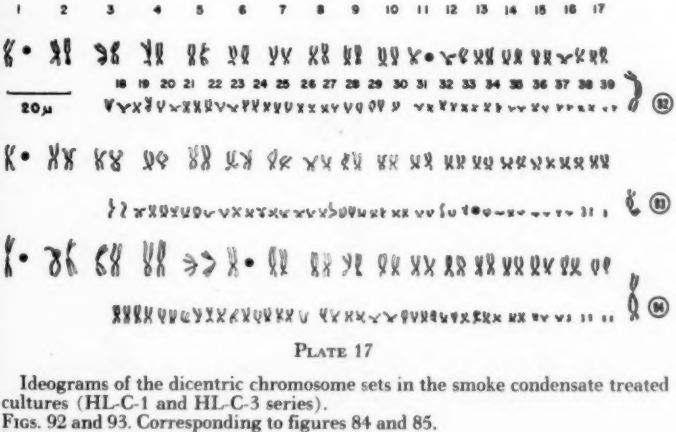
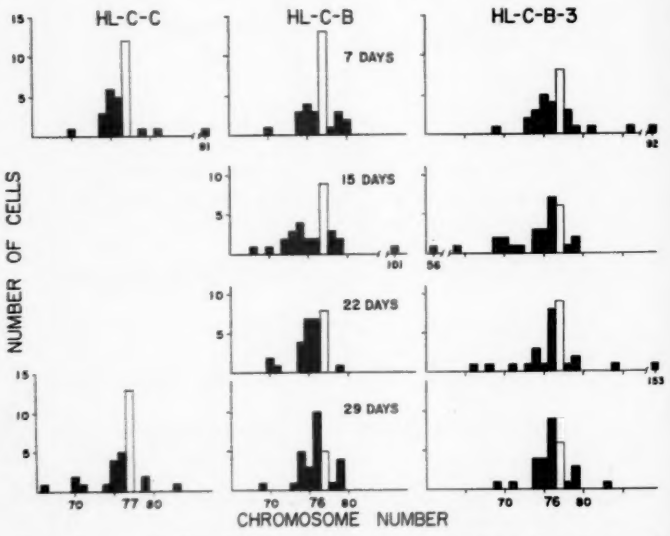


PLATE 17

Ideograms of the dicentric chromosome sets in the smoke condensate treated cultures (HL-C-1 and HL-C-3 series).
FIGS. 92 and 93. Corresponding to figures 84 and 85.
FIG. 94. Corresponding to figure 87.

the period of the experiment the most frequent chromosome number was 77 for untreated cultures which was identical with that obtained in the previous experience. Furthermore, in the cultures of both HL-C-B and HL-C-B-3 fixed on the 7th day after treatment, the most frequent number remained at 77 (out of 30 cells 13 in the HL-C-B and 8 in the HL-C-B-3, respectively). However, on the 15th day (2nd passage) in the HL-C-B-3, two peak values were obtained, at 76 and 77 (7 and 6 out of 30 cells, respectively). In contrast, in the HL-C-B the modal chromosome number remained at 77. In the 3rd passage of both lines, cell growth of the HL-C-B-3 was retarded as compared with the HL-C-B line. Therefore, the 4th culture was made only of HL-C-B. At this time (22nd day) the cells of the HL-C-B as well as elements from the 3rd passage of the HL-C-B-3 were fixed for chromosomal studies. The most frequent chromosome numbers of the HL-C-B were 75, 76, and 77 (7, 7, and 8 out of 30 cells, respectively), the HL-C-B-3 being 76 and 77 (8 and 9



TEXT-FIGURE 11. Distribution of chromosome numbers in cells treated with benzpyrene alone and with benzpyrene plus the #3 fraction of Neal's smoke condensate together with corresponding control. Based on 30 metaphase figures for each passage.

PLATE 18

Camera lucida drawings of the dicentric chromosome sets in the cells treated with benzpyrene alone (HL-C-B series) and with benzpyrene plus the #3 fraction of Neal's smoke condensate (HL-C-B-3 series).
FIG. 95. 76 chromosomes in the 4th passage of the HL-C-B.
FIG. 96. 56 chromosomes in the 2nd passage of the HL-C-B-3.

out of 30 cells, respectively). The latest chromosome counts of both lines on the 29th day showed shifts in the modal value. The most frequent chromosome number fell to 76 in the 5th passage of the HL-C-B and in the 4th passage of the HL-C-B-3. Furthermore, dicentric chromosomes appeared under both experimental conditions (Table 6 and Figs. 95, 96). However, their first appearance in the HL-C-B-3 series was 7 days earlier than for those of the HL-C-B, and 15 days earlier than those in cells treated with the No. 3 fraction alone.

Discussion

As reviewed recently by Cornfield et al (4), one of the links in the total evidence for the causal relationship of cigarette smoking and lung cancer is the demonstration that tobacco-smoke condensates have the biologic property of evoking carcinomas in certain laboratory animals, particularly in mice. The production of skin cancer in mice, following repeated, long-term applications of tobacco tar, has been reported by several workers (2, 7, 10, 28, 31, 36). The presence of carcinogenic materials in tobacco-smoke condensate was demonstrated by Gellhorn (10) and by Bock and Moore (2). Rockey and his associates (32) applied tobacco "tar" directly to the bronchial mucosa of dogs. Within 3 to 6 weeks, the tar-treated surface became granular and later developed wart-like elevations. Upon microscopic examination, hyperplasia, transitional metaplasia and squamous metaplasia were found in these areas. Leuchtenberger et al (19) exposed mice to cigarette smoke for a period up to 200 days. The bronchial epithelium was then examined microscopically. Bronchitis, basal-cell hyperplasia and atypical basal-cell hyperplasia were found in the majority of the animals while a few developed squamous metaplasia.

As far as we are aware, however, no attempt has been made to study the chromosomal changes in the cells after treatment with smoke condensate applying methods for cell culture. The present study was undertaken with the hope of determining whether constituents of tobacco-smoke condensates might be shown to alter the rate or sequence of events in the course of the establishment of cell lines from pulmonary tissue and whether

the development of any peculiar chromosomal injury could be associated with the presence of these chemicals in the medium. From these points of view this discussion will be concentrated on chromosomal changes following treatment with smoke condensates.

As has been described, the present observations on the chromosomes are divided into two groups, chromosomal changes in primary cultures and those in strain cell cultures. Since Painter (29) reported that the chromosome number for man was 48, and Oguma and Kihara (27) stated that there were 47 chromosomes in the male, a number of workers have challenged these counts. Hsu (11) reported 48 chromosomes in the material from tissue culture. However, he now states (12) that even with hypotonic solution treatment which greatly enhances the spreading of the chromosomes, his original observation was incorrect. Recently Tjio and Levan (34) and Tjio and Puck (35) found 46 chromosomes for cells of this species in tissue culture. Further, Kodani (16) reported that 46, 47 and 48 chromosomes occurred in testicular material. Nakanishi et al. (25) have studied the chromosome number of cells from human amnion tissue. In freshly isolated amnion cells before culture only 3 cells revealed chromosomes sufficiently well spread to permit accurate counting. The values obtained were 42, 48 and 55. Further, in primary cultures the most frequent chromosome numbers were 46 (25%) and 48 (21%). These values closely approximated those found in the course of the present study. Painter (30) discussed Kodani's suggestion that it seemed certain that humans might show either 46, 47 or 48 chromosomes, the variation in number being due to the presence of a definite chromosome pair, a univalent or the absence of an entire pair and pointed out that chromosome "fusion" was quite common in nature. In any case, the chromosome number for man seems to be in a diploid range from 46 to 48. However, in the cells treated with smoke condensate the most frequent chromosome numbers showed 43, 44, 45 and 46 on the 8th day. The shift of the modal values suggested chromosomal changes in shape. However, conspicuous changes were not observed between control and treated materials. Since it is well established that the range of aneuploidy increases in the course of in vitro life, the scattering of the chromosome numbers found

in the later observations might have been due to the long term cultivation even for cells in primary cultures.

Many workers reported that after continuous cultivation in vitro, cells of both normal and malignant origin exhibited extensive heteroploidy with very few or no diploids (cf., 1, 14, 15, 20, 21, 23, 25). Contrary to the above, Syverton (33) reported that in a strain of human tissue only diploids were found in many transfers. Recently Tjio and Puck (35) also stated that the chromosome number of cells from normal human skin, cervix and uterus remained constantly at 46 after more than 5 months of continuous, rapid growth in tissue culture involving scores of vessel transfers and a number of generations equivalent to many billions of progeny. Furthermore, in their studies on cells from the Chinese hamster Ford and Yerganian (9) found that the diploids coexisted with the heteroploids for a long period. The heteroploid transformation process of the chromosome numbers in the present study followed approximately the same pattern as that observed by Hsu et. al. (15), Hsu and Klatt (14) and Nakanishi (23); namely, the population at first showed only diploids, then widened its chromosomal spectrum to the stage of "great spread." Finally, a narrow spectrum of heteroploids was selected to represent the strain.

Since both control and treated lines (HL-C and HL-S strains) arose at approximately the same time, and also because the chromosome numbers of both lines showed the same pattern of scattering and finally, due to the fact that the modal values were established with no evidence of peculiarities in the shape of the chromosomes even in treated cultures, it is not believed that pretreatment with the smoke condensate was significantly important in the establishment of the strain.

The HL-C strain used in the present study has been shown to be a stable line in terms of its chromosome number and low aberration frequency. In a total of 450 metaphase figures of the HL-C-C (control) and HL-C-A (acetone control) in the present study, the most frequent chromosome number was constantly 77 for each observation from the 61st to 81st subcultures (during 95 days). Nakanishi and Mizutani (unpublished) have also observed the chromosome numbers of the mixed cultures of the HL-C and KL strains. The KL strain was established from a

4-week-old kitten lung tissue in this laboratory, and the most frequent chromosome numbers were 90 (22%) and 92 (26%) in the 84th subculture which was 411 days from the date of the primary culture (23). In the early passages after mixing the two strains the most frequent chromosome numbers showed two peaks at 77 (26%) and 90 (14%). However, in the 5th (30 days after mixing) and 10th (60 days) passages the chromosome number showed a single peak at 77 (48 and 46%, respectively). Therefore, it was presumed that the stemline cells with 77 chromosomes remained in this mixed culture and that the HL-C strain was the stable line in chromosome number.

After treatment with the smoke condensate as well as with benzpyrene alone and with a combination of smoke condensate and benzpyrene, the most frequent chromosome number fell to 76. In addition, in the period during which the change of the chromosome number was obtained, the occurrence of dicentric chromosomes would suggest a chromosomal rearrangement associated with establishment of a new stemline which had a different modal value. Nakanishi and Mizutani (in preparation), in the course of a study of chromosomal changes in successive subcultures of the Fernandes human amnion strain (8) surviving gamma irradiation from a $Cobalt^{60}$ source, found the most frequent chromosome number to be 75 (62%) in a range from 69 to 78 in the control (25). On the other hand, in the first passage which was set up from the irradiated culture at a dosage of 1,000r the chromosome numbers were distributed over a wider range from 60 to 135 with 5 small peaks at 71, 72, 73, 74 and 75. In this culture dicentric chromosomes appeared in 10% of the cells. Furthermore, in the 3rd passage (35 days after irradiation), the most frequent chromosome number was 73 (42%), and the dicentric chromosomes were no longer present. Thus, approximately the same process of chromosome number transformation as that which developed as a result of treatment with the smoke condensate was found. These data strongly support our interpretation of the occurrence of dicentric chromosomes as an explanation for the change of the chromosome number in the present study.

Koller (17) observed dicentric chromosomes in a rat tumor induced by an aromatic nitrogen mustard. The number, length

and shape of the dicentric bridges showed a very wide range of variation, and they were broken at anaphase or, if not broken, they gave rise to multiple nuclei and spindles. In order to study the behavior of dicentrics in living cells, tissue cultures of this tumor which had 14–18% of cells with anaphase bridges were made and the whole cycle of mitosis in several tumor cells was recorded by filming. However, no such cells were found either in the living or fixed cultures (18). They suggested that in tissue culture the conditions were not favorable for the cells with dicentrics either to survive or to proliferate. Koller (17) stated that the cause of the dicentric must be due to a metabolic disturbance. There are examples of "spontaneous" or natural chromosome fragmentation which are attributed to metabolic disturbances caused by environmental factors (5). Darlington and Wylie (6) also reported that a triploid *Narcissus* produced occasional dicentric chromosomes in its pollen grains.

Chu and Giles (3) found several cells with dicentric chromosomes in the HeLa strain (S3-RA1), and the morphology of this dicentric appeared to be the same in all 7 cells which were analyzed. They suggested that this presumably meant that the dicentric originally arose as a result of a single reciprocal translocation and was transmitted from this cell to some of its progeny. Hsu and Klatt (13) also reported that cells of strains L-P55 and L-P55-N2 of murine origin contained 1 or 2 long dicentric chromosomes. They considered two possibilities of the occurrence of dicentric chromosomes: 1) the dicentric translocation took place after the rest of the changes had been completed, and 2) all the cells originally had the dicentric but a part of them lost this peculiar chromosome due to its misbehavior during mitotic division. The first possibility seemed to be more plausible because none of the cells in Substrain L-P55-N2 lost this element after 6 months of continuous cultivation. Furthermore, the fact that the dicentric always retained its characteristic length and arm proportions also suggested that breakage and reunion of the bridge did not occur frequently, or whenever it happened, the daughter cells were not able to compete with the "standard" types and were thus eliminated from the population.

Recently Makino and Sasaki (22) reported that in a comparative ideogram study of the Yoshida sarcoma and its 4 subline derivatives each subline was characterized by its specific stemline

ideogram, which was clearly distinguishable from the others by the presence of 2, 3 and 4 V-shaped chromosomes of prominently large size, as well as from the stock tumor which was characterized by one such V chromosome. It is of particular importance to find that subline D, which is characterized by the presence of 4 V chromosomes, was derived from subline C with 3 V-types by intraperitoneal inoculation of refrigerated tumor cells after cold storage at −2° or −1°C. for 6 days. However, they stated that the origin of the large V-shaped chromosomes due to a centric fusion between two rod-shaped elements through translocation was merely hypothetical, since there was no positive morphological proof for its explanation, while speculation involving the nondisjunction of the V element was applied with a slight morphological support to one of the V's occurring in sublines C and D.

According to the investigations which have been reviewed, it appears possible that the so-called stemline cell which is characterized by having a definite ideogram and chromosome number may produce a new stemline under certain circumstances. In not only the strain culture but also in the primary culture there was a shift in the modal values toward lower chromosome numbers (present study). In the medium containing the smoke condensate as well as benzpyrene the cells might become adapted to the changed chemical environment. The following explanation is suggested: some stemline cells with the modal value of the chromosome number which is supposed to occupy the major part in the cell population could not divide regularly and the number of the cells with the modal value decreased in the new environment. Therefore the distribution of the chromosome numbers showed a wider range in the beginning of the treatment or of the adaptation to the new environment as a result of sampling of a certain number of metaphase figures. Some cells with the modal value then adapted to the environment and served anew as the primary contributors to growth of the population. However, these cells had unstable chromosome numbers as a result of the continuous or repeated effect of the smoke condensate. In such a period of transition dicentric chromosomes which appeared in some cells may not have migrated successfully to the poles at anaphase to produce the variable chromosome numbers in daughter cells. Finally, cells might adapt to

the changed environment gradually, and the new modal value become established in the cell population. Some chromosomal combinations might give the cells disadvantages or might even be lethal. Thus the new subline seemed to be the result of those cells which adapted to the challenging environment and which ultimately dominated the population.

Koller's observations (17) on the dicentrics in a rat tumor, as well as the report of Makino and Sasaki (22) regarding the presence of characteristic large V-shaped chromosomes in the Yoshida sarcoma and its subline derivatives, suggest a possible relationship between the existence of such peculiar chromosomes and specific malignant cells. However, even though the dicentric chromosomes appeared temporarily in some passages in the present study, it is reasonable to suppose the kind of relationship which has been suggested.

It will be necessary to recheck whether pretreatment with the smoke condensate is significantly important in the establishment of the strain and whether the chromosomal changes are associated with malignant conversions. For this purpose experimental animals such as rats, mice and Chinese hamsters rather than man would offer several advantages for cytological investigation as materials for tissue culture, since the chromosome numbers are lower and simpler in shape than those of the human and because the altered cells of such cultures could be implanted in the same species of animals to check their malignant potentialities.

Data collected from such studies might shed light on the vexatious question of the relationship between smoking and lung cancer.

Summary

Two epithelial cell strains were established from the lung of a two and a half month male human foetus. One of these was produced from cells in the presence of a smoke condensate (Ecusta) at a 1:10,000 dilution in Eagle's medium (HL-S) and another from an untreated control (HL-C). At the outset, the most frequent chromosome numbers showed a shift to lower values in treated cultures, then a widened chromosome spectrum developed to the stage of "great spread" in both treated and control cultures. Finally, a narrow peak of heteroploids became estab-

TABLE 9

Distribution of chromosome numbers in the cells treated with benzpyrene plus Neal's #3 fraction of smoke condensate (HL-C-B-3) in relation to culture passage

Passage Number	Chromosome Number																				
	56	64	66	68	69	70	71	72	73	74	75	76	77	78	79	81	83	84	86	92	>100
1 (7)*	..	..	..	..	1	..	..	..	2	3	5	4	8	3	1	1	..	..	1	1	..
2 (15)	1	1	..	..	2	2	1	1	..	3	3	7	6	1	2	..	..	..	..	..	..
3 (22)	..	..	1	1	..	..	1	..	1	3	1	8	9	1	2	..	..	1	..	..	1
4 (29)	..	..	..	..	1	..	1	..	..	4	4	9	6	1	3	..	1	..	..	..	..

* Lapse of time in days.

lished at 77 (HL-C) and 76 (HL-S). The HL-C strain was challenged with Neal's No. 1 and No. 3 fractions of smoke condensate together with acetone and untreated controls, as well as with benzpyrene alone and with benzpyrene plus the No. 3 fraction. In the two controls in each changed environment the modal value was constantly at 77 for each observation from the 61st to the 81st subcultures (during 95 days). In contrast, the new modal value of 76 chromosomes was established after the appearance of dicentric chromosomes in some passages.

Acknowledgments

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REFERENCES

1. Berman, L., C. S. Stulberg, and F. H. Ruddle: Human cell culture. Morphology of the Detroit strains. Cancer Res. 17: 668–676, 1957.
2. Bock, F. G., and G. E. Moore: Carcinogenic activity of cigarette-smoke condensate. I. Effect of trauma and remote X-irradiation. J. Nat. Cancer Inst. In press.
3. Chu, E. H. Y., and N. H. Giles: Comparative chromosomal studies on mammalian cells in culture. I. The HeLa strain and its mutant clonal derivatives. J. Nat. Cancer Inst. 20: 383–401, 1958.
4. Cornfield, J., W. Haenszel, E. C. Hammond, A. M. Lilienfeld, M. B. Shimkin, and E. L. Wynder: Smoking and lung cancer: Recent evidence and a discussion of some questions. J. Nat. Cancer Inst. 22: 173–203, 1959.
5. Darlington, C. D., and M. B. Upcott: Spontaneous chromosome change. J. Genetics 41: 297–338, 1941.
6. Darlington, C. D., and A. P. Wylie: A dicentric cycle in *Narcissus*. Symposium on chromosome breakage. Part III. Secondary and spontaneous breakage. 197–213. Springfield, Charles C. Thomas, 1953.
7. Engelbreth-Holm, J., and J. Ahlmann, Production of carcinoma in ST/Eh mice with cigarette tar. Acta. path. et microbiol. scandinav. 41: 267–272, 1957.
8. Fernandes, M. V.: The development of a human amnion strain of cells. Texas Rep. Biol. and Med. 16: 48–58, 1958.
9. Ford, D. K., and G. Yerganian: Observations on the chromosomes of Chinese hamster cells in tissue culture. J. Nat. Cancer Inst. 21: 393–425, 1958.
10. Gellhorn, A.: The cocarcinogenic activity of cigarette tobacco tar. Cancer Res. 18: 510–517, 1958.
11. Hsu, T. C.: Mammalian chromosomes *in vitro*. I. The karyotype of man. J. Hered. 43: 167–172, 1952.

12. Hsu, T. C.: Numerical chromosomal variations in higher animals. In: Developmental Cytology (Rudnick, D., ed.). New York, Ronald Press Co. 1959, Chap. 3, pp. 47–62.
13. Hsu, T. C., and O. Klatt: Mammalian chromosomes *in vitro*. IX. On genetic polymorphism in cell populations. J. Nat. Cancer Inst. 21: 437–473, 1958.
14. Hsu, T. C., and O. Klatt: Mammalian chromosomes *in vitro*. X. Heteroploid transformation in neoplastic cells. J. Nat. Cancer Inst. 22: 313–339, 1959.
15. Hsu, T. C., C. M. Pomerat, and P. S. Moorhead: Mammalian chromosomes *in vitro*. VIII. Heteroploid transformation in the human cell strain Mayes. J. Nat. Cancer Inst. 19: 867–873, 1957.
16. Kodani, M.: Three chromosome numbers in Whites and Japanese. Science 127: 1339–1340, 1958.
17. Koller, P. C.: Dicentric chromosomes in a rat tumor induced by an aromatic nitrogen mustard. Symposium on Chromosome Breakage. Part III. Secondary and spontaneous breakage. 181–196. Springfield, Charles C. Thomas, 1953.
18. Koller, P. C., and C. Waymouth: Observations on intracellular leucocytes in tissue cultures of a rat tumor. J. Roy. Mic. Soc. 72: 173–176, 1952.
19. Leuchtenberger, C., P. F. Doolon, and R. Leuchtenberger: A correlated histological, cytological, and cytochemical study of the tracheobronchial tree and lungs of mice exposed to cigarette smoke. I. Bronchitis with atypical epithelial changes in mice exposed to cigarette smoke. Cancer 2: 490–506, 1958.
20. Levan, A.: Chromosome studies on some human tumors and tissues of normal origin, grown *in vivo* and *in vitro* at the Sloan-Kettering Institute. Cancer 9: 648–663, 1956.
21. Levan, A., and J. J. Beisele: Role of chromosomes in cancerogenesis, as studied in serial tissue culture of mammalian cells. Ann. N. Y. Acad. Sc. 71: 1022–1053, 1958.
22. Makino, S., and M. Sasaki: Cytological studies of tumors. XXI. A comparative ideogram study of the Yoshida sarcoma and its subline derivatives. J. Nat. Cancer Inst. 20: 465–488, 1958.
23. Nakanishi, Y. H.: The development of a kitten lung cell strain and its chromosomes. Zeit. f. Zellforsch. In press.
24. Nakanishi, Y. H., and M. Mizutani: Observations on the somatic chromosomes of lamb cells in culture. Texas Rep. Biol. and Med. 17: 134–141, 1959.
25. Nakanishi, Y. H., M. V. Fernandes, M. Mizutani, and C. M. Pomerat: On the chromosome numbers of human amnion cells in primary and strain cultures. Texas Rep. Biol. and Med. 17: 345–353, 1959.
26. Neal, J.: Personal Communication.
27. Oguma, K., and H. Kuhara: Etude des chromosomes chez l'homme. Archives de Biologie 33: 493–514, 1923.
28. Orris, L., B. L. VanDuuren, A. I. Kosak, N. Nelson, and F. L. Schmitt: The carcinogenicity for mouse skin and the aromatic hydrocarbon content of cigarette-smoke condensate. J. Nat. Cancer Inst. 21: 557–561, 1958.
29. Painter, T. S.: Studies in mammalian spermatogenesis. II. The spermatogenesis of man. J. Morph. 37: 291–336, 1923.
30. Painter, T. S.: Post-graduate seminar, University of Texas, 1958.

31. Passey, R. D.: Carcinogenicity of cigarette tars. Brit. Empire Cancer Campaign. Thirty-fifth Annual Report 1957, pp. 65–66.

32. Rockey, E. E., M. Kuschner, A. I. Kosak, and E. Mayer: The effect of tobacco tar on the bronchial mucosa of dogs. Cancer 11: 466–472, 1958.

33. Syverton, J. T.: Comparative studies of normal and malignant cells in continuous culture. Spec. Publ. New York Acad. Sci. 5: 331–340, 1957.

34. Tjio, J. H., and A. Levan: The chromosome number of man. Hereditas 42: 1–6, 1956.

35. Tjio, J. H., and T. T. Puck: Genetics of somatic mammalian cells. II. Chromosomal constitution of cells in tissue culture. J. Exp. Med. 108: 259–268, 1958.

36. Wynder, E. L., E. A. Graham, and A. B. Croninger: Experimental production of carcinoma with cigarette tar. Cancer Res. 13: 855–864, 1953.

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LOCALIZATION OF THE AMINO-ACID OXIDASES AND THE GLUTAMIC-OXALACETIC TRANSAMINASE IN THE RAT KIDNEY

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Introduction

Studies on the localization of enzymes in the kidney were restricted to a few enzymes only, primarily because of the complicated structure of this tissue. It was shown by Mustakallino and Telkka (1953) and by Rennels and Ruskin (1954) that the succinic dehydrogenase is localized in the proximal convoluted tubules of the kidney, and that only proximal convoluted tubules contain alkaline phosphatase (Bunting 1948; Manheimer and Seligman, 1949). The amine oxidase and decarboxylase seem to be localized in the distal convoluted tubules (Oster and Schlossmand, 1942) and esterase (with indoxyl acetate as substrate) seems to be absent in the deep loops of Henle and collecting ducts (Barnett and Seligman, 1951).

In the present paper we tried to establish the localization of amino acid oxidases and oxacetic-glutamic transaminase in the cortex, outer medulla and inner medulla of the rat kidney.

Materials and methods

The Holtzman strain male albino rats, weighing 130 to 350 gm., were used. Rats were weighed and killed by a blow on the neck, and the kidneys immediately removed and immersed in ice-cold physiological solution. After decapsulation, the cortex, outer and inner medullas and the papilla were separated by dissection under the binocular microscope.

The cortex could be easily distinguished from the medulla by presence of the glomeruli. Poor vascularization enabled us to distinguish and thus separate the inner medulla from the inner zone of the outer medulla. Fresh tissues from the three different portions were then weighed and homogenized in double-distilled water. Thus, the kidney was separated into these three zones and each measured for its enzymatic activity (Fig. 1).

The amino-acid oxidase activity was estimated in each portion by measuring the amount of the ammonia formed after a period of 1 hour incubation at 38°C with various substrates. Ammonia was measured by the Conway diffusion method (Conway, 1950). The digest consisted of 1 ml of 10% homogenate of each portion of the kidney, 1 ml of pyrophosphate buffer of

Warburg and Christian (1937–38) and 0.5 ml of D-alanine, L-alanine or DL-alanine as substrates in 0.01M concentrations. The mixture of 1 ml. of the pyrophosphate buffer was used as a blank. The reaction was stopped by the addition of 0.5 ml of 5% trichloracetic acid and adjusted to pH 7 by a weak solution of NaOH.

For ammonia estimation 1.0 ml digest was placed in the outer chamber of the Conway cell and 500 lambda of 0.0010 N hydrochloric acid mixed with

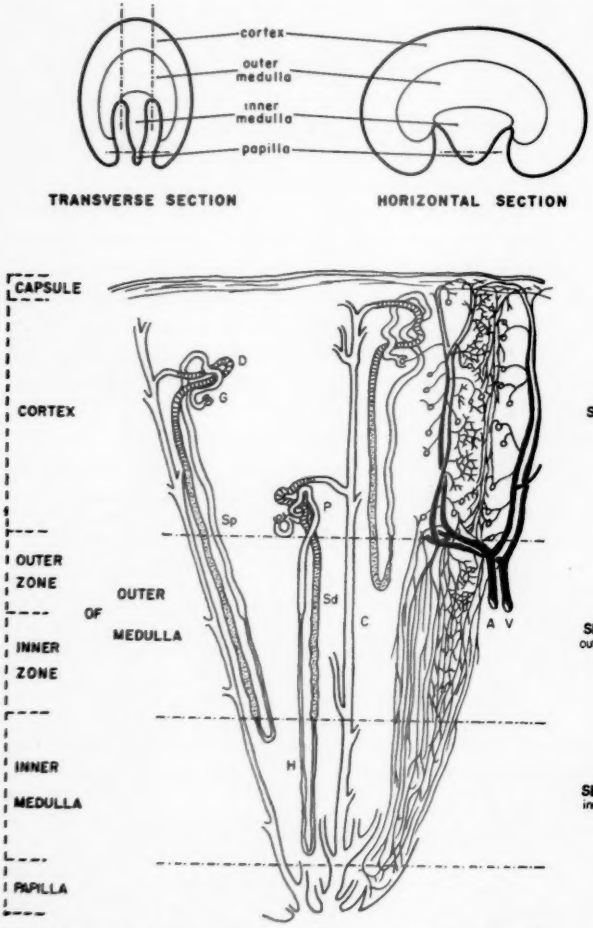

FIG. 1. Method of sectioning the rat kidney for histochemical analysis.

Tashiro's indicator (Tashiro, 1922) were added to the inner chamber. One ml. of ammonia free saturated potassium carbonate was then placed in the outer chamber. After mixing the solutions in the outer chamber, the cells were allowed to stand at room temperature for exactly 30 minutes, and the content of the inner chamber was immediately titrated with 0.010 N NaOH, using a Kirk ultra-microburette. As endpoint the complete disappearance of the reddish color or the first appearance of a very faintly perceptible green was taken. The diffusion factor of the kidney homogenate was found to be 2.0 as established by the Parnas and Heller's distillation method (Parnas and Heller, 1924).

For the calculation of the amount of ammonia, the following formula was used:

$$\frac{(B - A \times 2 \times a \times C}{(B - S) \times (\text{mg. of tissue per 1 ml. of homogenate})} = NH_3\ \mu g/\text{mg. tissue}$$

where, B: Titration of blank digest
A: Titration of experimental digest
S: Titration of control digest
2: Diffusion factor
a: Concentration of the control solution, 2 μl (μg./ml.)
C: Dilution factor of the homogenate.

Small amounts of tissue were taken from each portion to measure the percentage relation of dry weight to the fresh weight. Tissue were dried overnight in the oven at 102°C., weighed and the percentage of dry weight calculated.

Estimation of the deoxyribonucleic acid: The deoxyribonucleic acid (DNA) was estimated by Dische's diphenylamine method as worked out for small amounts of tissues by Nowinski and Yushok (1953). It was found that the DNA content per nucleus was 13.4×10^{-6} μg. and 100 μg. DNA correspond to the population of 7.5×10^6 cells in the kidney of the strain albino rat used (Unpublished Data). Enzymatic activity was expressed as suggested by Nowinski (1955) on the basis of mg. wet weight, mg. dry weight and the cell population.

For the estimations of glutamic-oxalacetic transaminase, homogenates were prepared with double-distilled water from the cortex, the outer medulla and the inner medulla. The amount of protein in each homogenate was measured by the biuret method. Activity of the transaminase (both directions of the reaction) was measured according to the Cammarata-Cohen (1951) spectrophotometric method. Because of small amounts of tissues, the concentrations of the homogenates had to be considerably more diluted than in the original method (1 mg. per ml). During preliminary experiments, it was found that such diluted homogenates showed higher activity than the non-diluted ones, when calculated on the 1 mg. protein basis. This might have been due to the fact that the concentration of the substrate was maintained unaltered. Therefore, the protein concentration of each homogenate was kept as closely as possible to the same levels in all experiments in order to compare the enzymatic activities under identical conditions (Fig. 2).

Results were calculated on the basis of 1 mg. protein (Q) and on 100 μg. DNA.

Results

First the percentage of dry weight to wet weight in each portion of the kidney was estimated and found to be the highest in the cortex: 19.9%; in the outer medulla 13.7% and in the inner medulla 10.2%. These estimations were carried out on 10 rats. For the calculation of results on the DNA-basis, the DNA-content per mg wet weight had to be established. On 10 rats it was shown that the DNA of the cortex was 66.5 μg ± 8.05, the outer medulla contained 72.7 μg ± 8.47 and in the inner medula 55.7 μg ± 9.71.

Knowing the amount of DNA which corresponds to 10^6 cells, it was easy to calulate the number of cells per mg in each portion of the kidney. The cortex contained 5×10^6 cells, the outer medulla 5.47×10^6 and the inner medulla 4.19×10^6 cells. The calculation of the t-value showed a high significance of difference between cortex and inner medulla ($t = 4.7$), but not between cortex and outer medulla ($t = 2.6$).

The amino-acid oxidase activity. The amino-acid oxidase activity was expressed by the amount of NH_3 in μg. on the different bases per one hour of incubation.

In Table I and Fig. 2 the activities of amino-acid oxidases in different parts of kidney on a wet weight basis are summarized. As substrates, D- and L-forms of alanine were added, as well as the racemic form. The concentrations of the substrates were 0.01 M throughout the experiment.

The racemic form of alanine showed the highest activity in the cortex (1.94 μg.), but was considerably lower in the outer and inner medullas (0.34 μg. and 0.26 μg., difference being not

TABLE 1

Activity of Amino-Acid Oxidase expressed in μg of NH_3 per mg wet weight with Standard Deviations of the mean averages of kidneys from 6 rats

	DL-alanine	D-alanine	L-alanine
Cortex	1.94 ± 0.570	3.61 ± 0.683	0.03 ± 0.031
Outer medulla	0.34 ± 0.081	0.19 ± 0.116	0.03 ± 0.018
Inner medulla	0.26 ±0.091	0.08 ± 0.024	0.05 ± 0.013

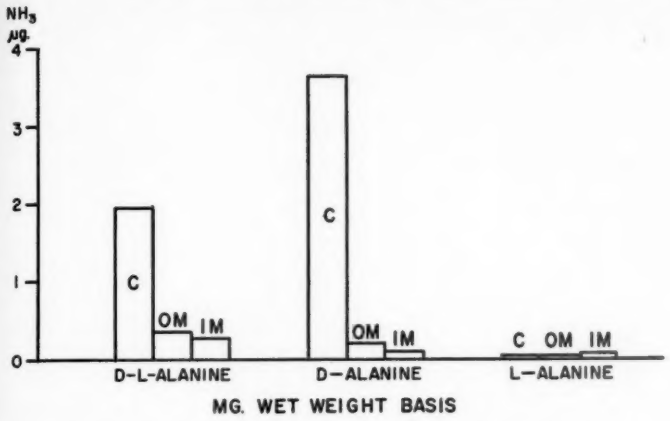

FIG. 2. Activity of Amino-acid oxidases expressed in μg NH_3 produced per hour per mg wet weight. C = Cortex; OM = Outer Medulla; IM = Inner Medulla.

significant). The D-alanine showed essentially the same distribution pattern as the racemic form and the differences of the activities between the outer and inner medullas were insignificant. The L-alanine, however, showed extemely low activity and which did not change in all the three zones (0.03 μg., 0.03 μg. and 0.05 μg.; difference not significant).

On the dry weight basis (Table II, Fig. 4) the racemic form and the D-alanine had practically the same distribution as on the wet weight basis, i.e., the highest activity was shown by the cortex (10.23 μg. and 18.15 μg., respectively) and lower activity by both the medullas (2.16 μg. and 2.60 μg.; 1.36 μg. and 0.80 μg., differences not significant in each case). The L-amino-acid oxidase, however, showed the highest activity in the inner medulla (0.53 μg.) and lower activities in the cortex and the outer medulla (0.16 μg. and 0.20 μg., difference insignificant).

On the cell population basis (100 μg DNA corresponds to 7.5×10^6 cells) distribution of the amino-acid oxidase activities (Table III Fig. 3) was similar to that obtained on the basis of dry weight. The highest activity of the racemic form and the D-

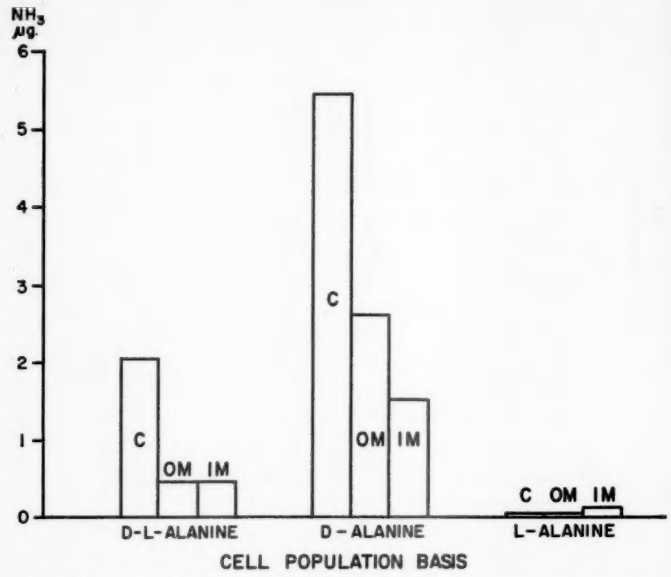

FIG. 3. Activity of Amino-acid oxidases expressed in μg NH_3 produced per 1 hour and 100 μg DNA 7.5×10^6 cells. C = Cortex; OM = Outer Medulla; IM = Inner Medulla.

TABLE 2

Activity of Amino-Acid Oxidase expressed in μg of NH_3 per mg dry weight with Standard Deviations of the mean averages of kidneys from 6 rats

	DL-alanine	D-alanine	L-alanine
Cortex	10.23 ± 2.612	18.15 ± 3.398	0.16 ± 0.142
Outer medulla	2.16 ± 0.518	1.36 ± 0.840	0.20 ± 0.127
Inner medulla	2.60 ± 0.977	0.80 ± 0.224	0.53 ± 0.100

alanine was shown by the cortex (2.92 μg. and 5.43 μg.) and that of the L-alanine by the inner medulla (0.10 μg.).

Glutamic-Oxalacetic Transaminase. Activity of the transaminase was expressed as Q (μl. of substrate transaminases per mg. protein per 1 hour) and on the cell population basis.

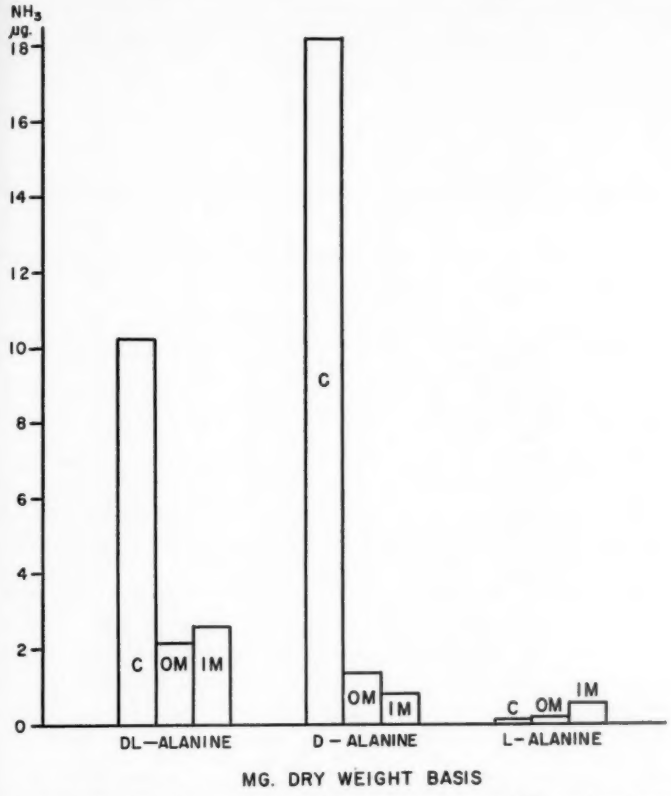

FIG. 4. Activity of Amino-acid oxidases, expressed in µg NH_3 produced per hour and per 1 mg dry weight. C = Cortex; OM = Outer Medulla; IM = Inner Medulla.

TABLE 3

Activity of Amino-Acid Oxidases calculated on cell population basis (100 µg DNA—7.5 × 10^6 cells)

In parenthesis number of rats used

	DL-alanine	D-alanine	L-alanine
Cortex	(7) 2.02 ± 0.903	(6) 5.43 ± 1.024	(6) 0.05 ± 0.042
Outer medulla	(7) 0.46 ± 0.142	(6) 0.26 ± 0.159	(6) 0.04 ± 0.027
Inner medulla	(7) 0.46 ± 0.163	(6) 0.15 ± 0.044	(6) 0.10 ± 0.022

TABLE 4

Transaminase Activity (Q) on 1 mg protein basis

Number of rats	8
Cortex	68.7 ± 2.13
Outer medulla	83.9 ± 2.00
Inner medulla	34.3 ± 3.16

TABLE 5

Transaminase Activity calculated on cell population basis (100 μg DNA = 7.5 × 10^6 cells)

Number of rats	8
Cortex	36.9 ± 1.67
Outer medulla	29.6 ± 1.55
Inner medulla	8.6 ± 1.81

On the Q-basis, as summarized in Table IV, the outer medulla showed the highest activity (83.9), the cortex had a significantly lower one (68.7) and the inner medulla still lower (34.4).

On the cell population basis, (Table V, Fig. 5) the cortex showed higher activity (36.9) than the outer medulla (29.6), this difference being significant. The lowest activity was shown by the inner medulla when calculated on the protein basis.

Discussion

On the μg per wet weight-dry weight and cell population basis, the D-amino-acid oxidase showed the highest activity in the cortex and lower activity in the outer and inner medullas. The results with L-amino-acid oxidase, however, were inconsistent as calculated on this basis. On the wet weight basis, there were no particular differences in its localization, but on the dry weight basis, the highest figures were obtained in the inner medulla. The same result was obtained when the data were calculated on the cell population basis.

The content of water and evaporable constituents, of fresh kidneys is influenced by so many factors that the wet weight often varies to a great extent. Therefore, even if two pieces of

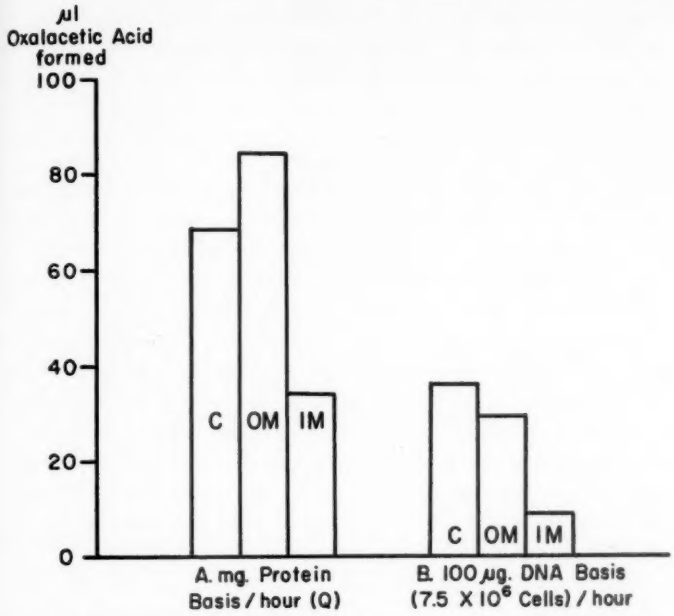

FIG. 5. Activity of Glutamic–oxalacetic transaminase. A. on 1 mg protein basis (Q); B. on 100 µg DNA basis. C = Cortex; OM = Outer Medulla; IM = Inner Medulla.

fresh tissue weigh exactly the same, they need not be identical from the standpoint of the actual amount of parenchyma, or the cells. The inner medulla has the lowest dry weight percentage (10.2%) and the least number of cells (4.19×10^6) of the three zones, which indicates that it is rich in water, most probably of urine. Hence the expression, enzymatic activity on the basis of wet weight, does not give the true figures. Compared to the cell population basis, the protein nitrogen basis (as expressed either on dry weight or 1 mg. protein basis) has also a disadvantage. Since it refers to unit protein nitrogen, any change which occurs in the content of proteins in cells or tissues, will influence the calculation of enzymatic activity. This might be the case with the discrepancy of the transaminase activity between the mg.

protein basis and the cell population basis, as seen in Table IV. When the wet or dry weight is used as reference, any intrinsic changes influences the standard of reference.

As stated by Thompson et al (1953), the best procedure is to use the DNA content of the tissue as a measure of cell number, like 100 μg DNA which correspond to 7.5×10^6 cells in the normal Holtzman strain albino rat kidney, and to use the ratios of the enzymatic activity to the DNA as a measure of average cell compositions. On the other hand, the calculation on the cell population basis, as emphasized by Nowinski and Yushok (1953) has the advantage of representing the average enzymatic activity of one population (say cortex) which can be compared to other populations (like outer and inner medullas). Cytological localization of the enzymes studied cannot be carried out at present, since no appropriate histochemical methods exist.

The urinary pH is primarily a function of the ammonia production; in all probability it serves to neutralize the acid residue left in tubules (Krebs, 1935). It has been shown that the ammonia formation takes place in the distal tubule and probably in the collecting duct (Walker, 1940–41). The majority of the distal tubules are located in the cortex and the fact that the highest activity of the D-amino-acid oxidase is found in the cortex, appears to correspond to the generally accepted theory.

There is a possibility that transaminase may play a minor role in NH_3 formation in the kidney through the system of transdeamination (Braunstein and Bychkov, 1939), in the relationship with L-glutamic oxidase. By and large, however, the role of glutamic-oxalacetic transaminase in the kidney needs further investigation.

Summary

1. The enzymatic activity of the amino-acid oxidases and glutamic-oxalacetic transaminase in the cortex, the outer medulla and the inner medulla of the normal albino rat kidney was studied.

2. The D-amino-acid oxidase has considerably higher activity than the L-amino-acid oxidase which is also in the kidney;

D-amino-acid oxidase is localized primarily in the cortex and L-amino-acid oxidase in the inner medulla.

3. The Q for glutamic-oxalacetic transaminase shows the highest activity in the outer medulla (on the 1 mg. protein basis), but on the cell population basis the highest activity is shown by the cortex.

REFERENCES

1. Barnett, R. J. and Seligman, A. M.: Histochemical Demonstration of Esterases by Production of Indigo. *Science*, 114:579, 1951.
2. Braunstein, A. E. and Bychkov, S. M.: A Cell-free Enzymatic Model of 1-Amino-acid Dehydrogenase. *Nature*, 144:751, 1939.
3. Bunting, H.: Kidney Alkaline Phosphatase of Rats Following Alloxan Induced Diabetes and Acute Hypo- and Hyper-glycemia. *Proc. Soc. Exptl. Biol. and Med.*, 67:370, 1948.
4. Cammarata, P. S. and Cohen, P. P.: Spectrophotometric Measurement of Transamination Reactions. *J. Biol. Chem.*, 193:45, 1951.
5. Conway, E. J.: *Microdiffusion Analysis and Volumetric Error*. 2nd edition, Crosby Lockwood & Son, Ltd., London, 1950.
6. Krebs, H. A.: Deamination of Amino Acids. *Biochem. J.*, 29:1620, 1935.
7. Manheimer, L. H. and Seligman, A. M.: Improvement in the Method for the Histochemical Demonstration of Alkaline Phosphatase and its use in a Study of Normal and Neoplastic Tissues. *J. Nat. Cancer Inst.*, 9:181, 1949.
8. Montgomery, H. and Pierce, J. A.: The Side of Acidification of the Urine within the Renal Tubule in Amphibia. *Am. J. Physiol.*, 118:144, 1937.
9. Mustakallio, K. K. and Telkka, A.: Histochemical Localization of the Mercurial Inhibition of Succinic Dehydrogenase in Rat Kidney. *Science*, 118:320, 1953.
10. Nowinski, W. W.: Some Metabolic Aspects of Multiplicative Growth. *Tex. Rep. Biol. and Med.*, 13:651–658, 1955.
11. Nowinski, W. W. and Yushok, W. D.: Establishment of Multiplicative Growth Rates on the Basis of Desoxyribosenucleic Acid. *Biochimica et Biophysica Acta*, 11:497, 1953.
12. Oster, K. A. and Schlossman, N. C.: Histochemical Demonstration of Amine Oxidase in the Kidney. *J. Cell. Comp. Physiol.*, 20:373, 1942.
13. Parnas, J. K. and Heller, J.: Uber den Ammoniakgehalt und über die Ammoniakbildung im Blute, I. *Biochem. Z*, 152:1, 1924.
14. Rennels, E. G. and Rushkin, A.: Histochemical Changes in Succinic Dehydrogenase Activity in Rat Kidney Following Administration of Mercurial Diuretics. *Proc. Soc. Exp. Biol. and Med.*, 85:309, 1954.
15. Tashiro, S.: Studies on Alkaligenesis in Tissues. *Am. J. Physiol.*, 60: 519, 1922.
16. Thomson, R. Y., Heagy, F. C., Hutchison, W. C. and Davidson, J. N.: The Deoxyribonucleic Acid Content of the Rat Cell Nucleus & its Use in Expressing the Results of Tissue Analysis, with Particular Reference to the Composition of Liver Tissue. *Biochem. J.*, 53:460, 1953.

17. Walker, A. M.: Ammonia Formation in the Amphibian Kidney. *J. Physiol.*, 131:187, 1940–41.

18. Warburg, O. and Christian, W.: Isolierung der prosthetischen Gruppe der d-Aminoseoxidase. *Biochem. Z.*, 298:150, 1938.

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SURGICAL REMOVAL OF HEARTWORMS BY OPEN CARDIOTOMY USING TOTAL VENOUS OCCLUSION*

H. S. POLLARD, JR., R. ASHBY, AND J. R. DERRICK[a]

The chemotherapy of heartworms (Dirofilaria immitis) in dogs has two disadvantages: pulmonary embolism (1, 2, 4, 5) resulting from therapy, and the toxicity of the large doses of arsenicals required (3).

This paper presents a surgical method of removing adult worms from the heart. Following surgery, the animal was readily cleared of microfilaria with small doses of arsenicals. The operation required little equipment (Fig. 1), and few personnel.

Method

The operating team consisted of three persons: the surgeon, one assistant, and either a third person or a machine to inflate the lungs while the chest was open.

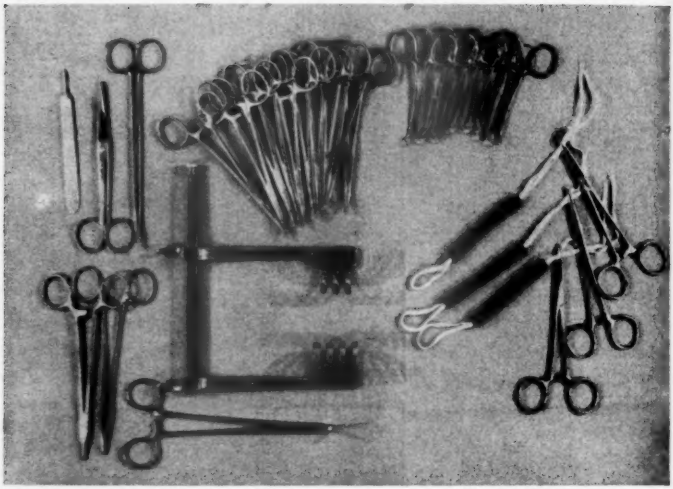
Veterinary Nembutal* (pentobarbital sodium) was selected as the anesthetic agent for two reasons: 1) simplicity of use, and 2) the prolonged sedation, simplifying postoperative management of the animals. Endotracheal intubation was necessary to allow pulmonary expansion during the thoracotomy.

A rubber anesthesia bag or suitable mechanical breathing device was attached to the endotracheal tube so that respirations might be maintained manually or mechanically while the chest was open. With the animal strapped on a dog board, right side up, the right side of the chest was sheared, shaved, and prepared aseptically by conventional means. Saline was infused into a saphenous vein.

A curved incision was made along the 5th right intercostal space. The shoulder gurdle muscles which overlie the interspace were carefully divided by incision. The dissection was continued to the intercostal muscle layer, all bleeding points being carefully clamped to minimize blood loss. Pure oxygen was administered for the remainder of the procedure.

The thoracic cavity was entered by division of the intercostal muscles in the 5th intercostal space. Exposure for the procedure was obtained by slowly opening a rib spreader (Fig. 1). Umbilical tapes were placed around the superior vena cava, inferior vena cava, and azygos vein at points prior to their entrance into the pericardial sac. The two loose ends of each tape were then passed through a one inch soft rubber tube (Figs. 1 and 2).

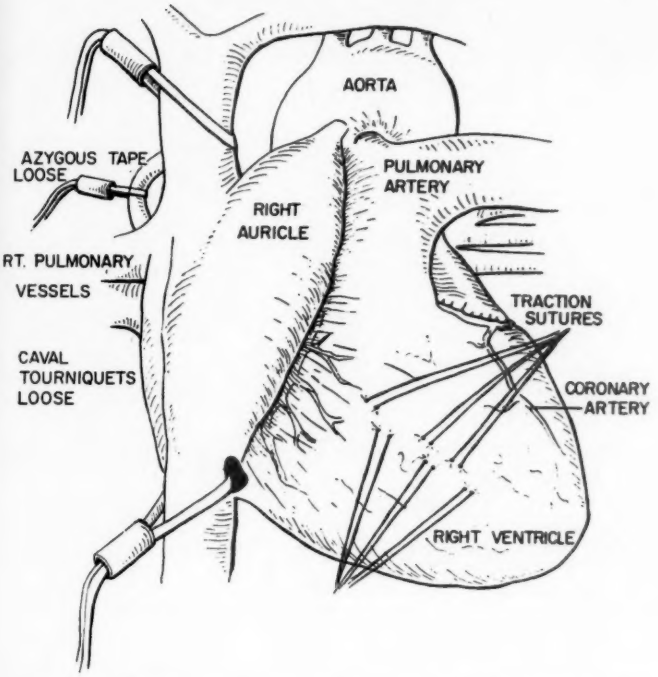
With all tapes in place, the pericardial sac was opened to expose the right ventricle. Two to four stay sutures were placed in the right ventricular muscle for use as retractors during the ventriculotomy (Figs. 2 and 3).



Before the ventriculotomy, the lungs were vigorously inflated for one minute while the surgeon arranged his instruments for quick availability during the period the heart would be open. At this point, the bottle of saline (dripping intravenously in the leg vein) was replaced by a 250 cubic centimeter bottle of citrated whole blood. 100 to 250 cubic centimeters of which were administered during the procedure. The tourniquets on the azygos vein and the inferior and superior cavae were tightened and the time noted. The anesthetist allowed the lungs to collapse; he then called out the total duration of occlusion each 30 seconds until the tapes had all been released.

A 3 to 5 centimeter incision was made in the right ventricle with scissors, parallel to the coronary arteries and between the stay sutures. The stay sutures were used to retract the edges of the incision for exposure (Fig. 3). By immediately sucking the blood out of the right ventricle, one could visualize the entire chamber and the pulmonary outflow tract. The adult heart worms, usually formed as a bolus of 8 to 15 worms, were grasped with forceps and removed from the heart. By traction on the stay sutures the surgeon drew together the edges of the ventricular incision and closed the ventriculotomy, using a continuous over and over suture of 0000 arterial silk (Fig. 4). Towel slips could be placed between the stay sutures if additional approximation of the ventricular edges was required to prevent excessive blood loss.

When the cardiotomy incision was approximately 70 per cent closed by the suture, the azygos tourniquet was released. This effectively cleared the ventricle of residual air and allowed the heart to accommodate to a gradual inflow of blood. Closure of the ventricle was then completed, the superior caval tourniquet released and the lungs re-expanded. A second row of con-

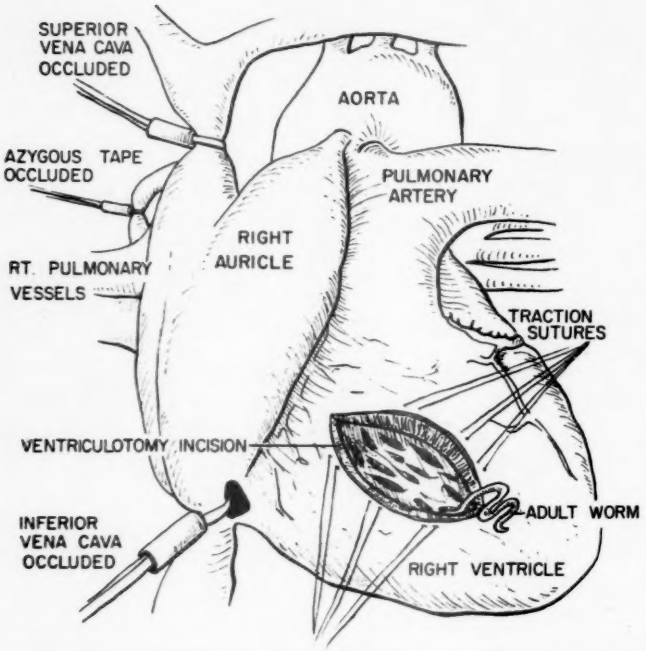


tinuous 0000 silk suture was begun. The tape on the inferior vena cava was released one minute after that on the superior cava.

The pericardium was closed with interrupted silk sutures placed one centimeter apart. A rubber thoracic drainage catheter was placed intercostally through a stab wound (Fig. 5) and the chest closed in 4 layers with continuous 00 chromic catgut. After closure of the intercostal layer, the catheter was connected to an underwater trap bottle (Fig. 5) to maintain expansion of the lungs.

The duration of total venous occlusion should not exceed three minutes or irreversible cerebral changes were likely to occur. This time limit was more than adequate for the removal of heart worms in nearly all cases. If additional time was required, however, the incision could be approximated by traction on the stay sutures, the occluding tapes released, and the blood recirculated and re-oxygenated for one minute. Blood loss during this maneuver was not excessive. Blood loss during the cardiotomy usually amounted to less than 100 cubic centimeters.

Supportive and Postoperative Management. All animals in our series were "poor risk" cases in very poor nutritional status.



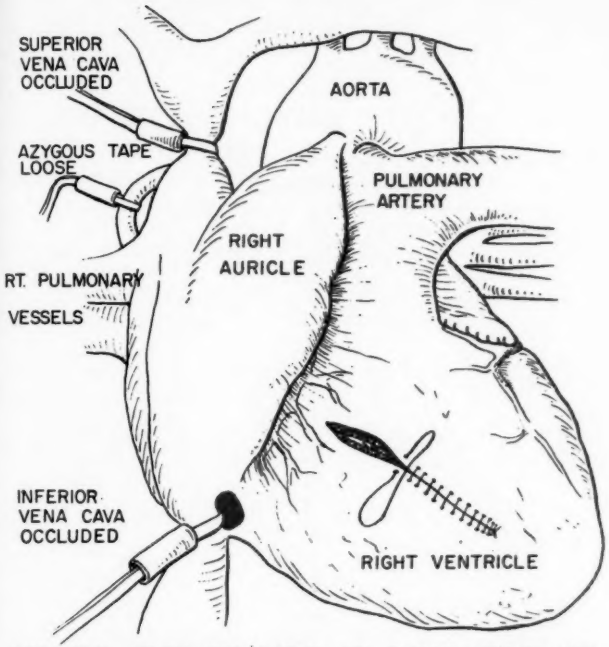
TOTAL VENOUS OCCLUSION DURING VENTRICULOTOMY

Postoperatively, while still sedated, the animals were given an intravenous infusion of 150 to 300 cubic centimeters of 5 per cent glucose in water containing 0.25 grams of Streptomycin, 300,000 units of aqueous penicillin and 2 cubic centimeters of a parenteral multivitamin preparation.

The intercostal catheter was left in place until postoperative drainage was minimal (usually 1½ to 2 hours). During this period, the animals were turned frequently from side to side to prevent atelectasis and aid in expansion of the lungs. Postoperative digitalization was not necessary in any of the dogs.

Results

Eight dogs have undergone right ventriculotomy with total venous occlusion. One death occurred on the fourth postoperative

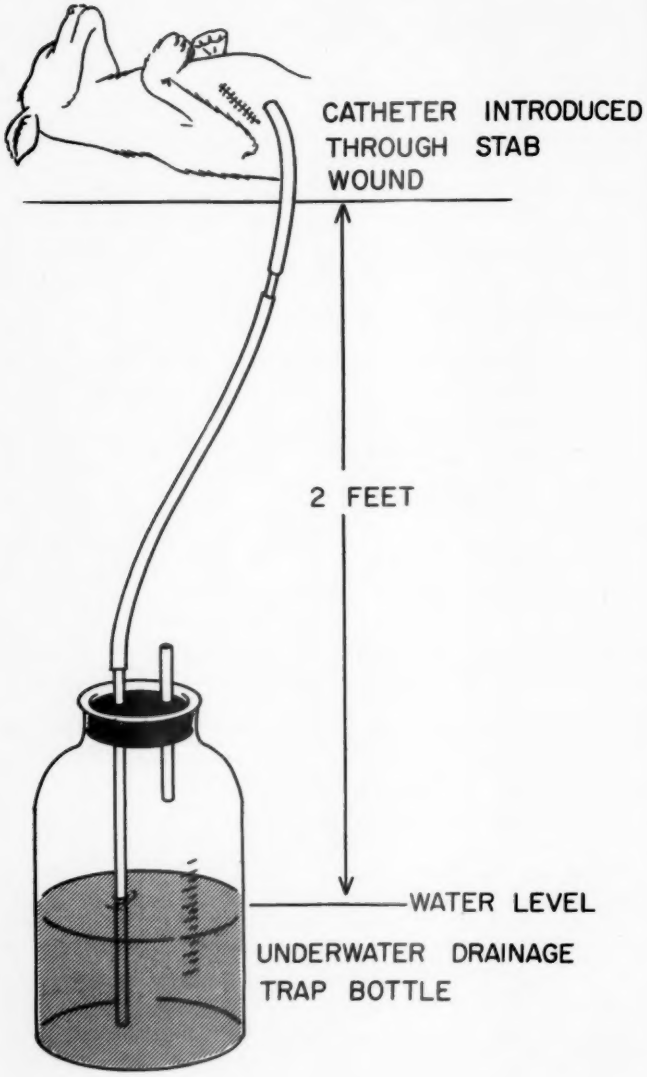


AZYGOUS TAPE RELEASED TO CLEAR HEART OF AIR BEFORE COMPLETE CLOSURE OF VENTRICULOTOMY

day, due to pulmonary complications in a dog which aspirated gastric contents before tracheal intubation. (The animal was fed immediately before operation by a new employee in the laboratory.) No operative deaths occurred. The animals were observed from one to three weeks postoperatively and were then sacrificed, all dogs demonstrating good healing at the ventriculotomy site and a complete absence of heart worms.

Summary

Adult Dirofiliaria worms were surgically removed by a technique involving total venous occlusion. The superior and inferior vena cavae and azygos vein were occluded with umbilical tape for as long as three minutes, additional time being obtained, if necessary, by a brief recirculation maneuver. Cardiotomy was


CATHETER INTRODUCED
THROUGH STAB
WOUND
2 FEET
WATER LEVEL
UNDERWATER DRAINAGE
TRAP BOTTLE

performed and the worms removed with forceps. Eight dogs have successfully undergone this procedure.

REFERENCES AND NOTES

1. Hays, I. M., Some Observations on Filariasis and Its Treatment. *J.A.V.M.A.*, 86:55, 1935.
2. Kume, S., Ohishi, I., Observations on the Chemotherapy of Canine Heartworm Infection with Arsenicals. *J.A.V.M.A.*, 131:476, 1957.
3. Kume, S., Chemotherapy of Canine Filariasis. *A. J. Vet. Research*, 18:912, 1957.
4. Soltys, A., Heartworms in the Dog. *J.A.V.M.A.*, 129:520, 1956.
5. Turk, R. D., Gaafar, S. M., and Lynd, F. T., A Note on the Occurrence of the Nematodes, *Dirofilaria Immitis* and Ancylostoma Braziliense in Unusual Locations. *J.A.V.M.A.*, 129:424, 1956.

* Courtesy of Abbott Laboratories, North Chicago, Illinois.

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EGG PRODUCTION OF *NEMATOSPIROIDES DUBIUS* IN MICE AND RATS*

J. ALLEN SCOTT, JOHN H. CROSS, JR. AND CHARLTON DAWSON

The laboratory mouse has been shown to act as a normal host for *Nematospiroides dubius*, a widely distributed parasite of microtine rodents (Spurlock, 1943; Baker, 1954; Ehrenford, 1954; and Cross, 1958). Although the laboratory white rat has a complete natural resistance to this parasite, Cross (1959) has shown that this resistance can be overcome if the infection is given while the rats are under daily treatment with cortisone. Under these conditions the worms develop to the egg-laying stage in rats essentially as they do in mice. The larvae hatched from the eggs have the same degree of infectivity as do those from mice, but the period of egg production is much shorter than in mice.

The present study was undertaken for three purposes: (1) to determine quantitatively the complete cycle of egg production throughout the life of the worms in mice, (2) to determine whether the egg production level differed in the two hosts, and (3) whether the curtailed period of egg output in rats was caused by premature death of worms or by a shorter reproductive period. A preliminary report of these studies was presented by Cross et al., 1958.

Methods and materials

The strain of worms used for these experiments, and the methods of culturing and administering larvae were the same as those described by Cross (1959). The larvae were administered by depriving the animals of water for short periods and then allowing them to drink, from a pipette, water containing known numbers of larvae. The mice were of the CF-1 and C3H strains, and the rats of the CFW strain. No differences between strains of mice were detected. Cortisone acetate was given subcutaneously to the rats in amounts of 5 mg per 100 grams body weight each day from the day of infection until the appearance of eggs.

The egg counts for rats were made by modifications of the procedure described by Stoll and Hausheer (1926). The total fecal output was collected and weighed for each 24 hour period. After thorough stirring a 4 ml sample was measured into 56 ml of N/10 NaOH in a displacement flask. When the

fecal material was thoroughly dispersed in the solution by alternate standing and shaking, a 0.075 ml portion was removed with a pipette described by Scott (1932), placed on a wide slide and covered with a 22 × 30 mm cover glass. Eggs in the portion extending beyond the edge of the cover glass were first counted and then those underneath were counted by use of a mechanical stage. The resulting count multiplied by the dilution factor of 200 and by the 24 hour fecal weight was used as an estimate of the daily egg output. For mice the total 24 hour output of feces was diluted to 60 ml in a displacement flask. A count, made as before, and multiplied by a dilution factor of 800 was used as an estimate of the daily output.

Results

Egg production cycle in the mouse. The first experiment was designed to follow the egg output throughout the life of the infection. Three young, mature mice were infected with 100 to 300 larvae. Counts were made daily from the 12th to the 35th day of infection. Since by then the output had risen to a peak and levelled off, counts were made at 3-day intervals until the 88th day after which they were continued at weekly intervals. One mouse died on the 125th day and 22 male and 49 female worms were recovered at autopsy. Another mouse died on the 170th day, but was not found immediately and the 20 male and 32 female worms were dead when recovered at autopsy. The third mouse died on the 282nd day and no worms were found at autopsy.

The egg output data from these three mice are summarized in Figure 1. The daily counts are plotted until after the first peak, then counts made at 3-day intervals until the 85th day, after which the plotted points represent, for the most part, counts made at weekly intervals. The third mouse, whose counts are plotted only to the 220th day, had counts of one egg per slide, i.e., 800 eggs per day, on the 233rd, 247th, 272nd and 276th days. The method of plotting was chosen to present a visual image of the variability. Although daily counts were made through the 35th day, they could not be plotted on a scale suitable for reproduction of the entire series. Running averages were used to study the trends, but they did not show any essential differences from those evident in the figures shown here. Coefficients of variation of the daily counts calculated from the period from the 15th through the 35th days were 21, 22, and 20 per cent respectively.

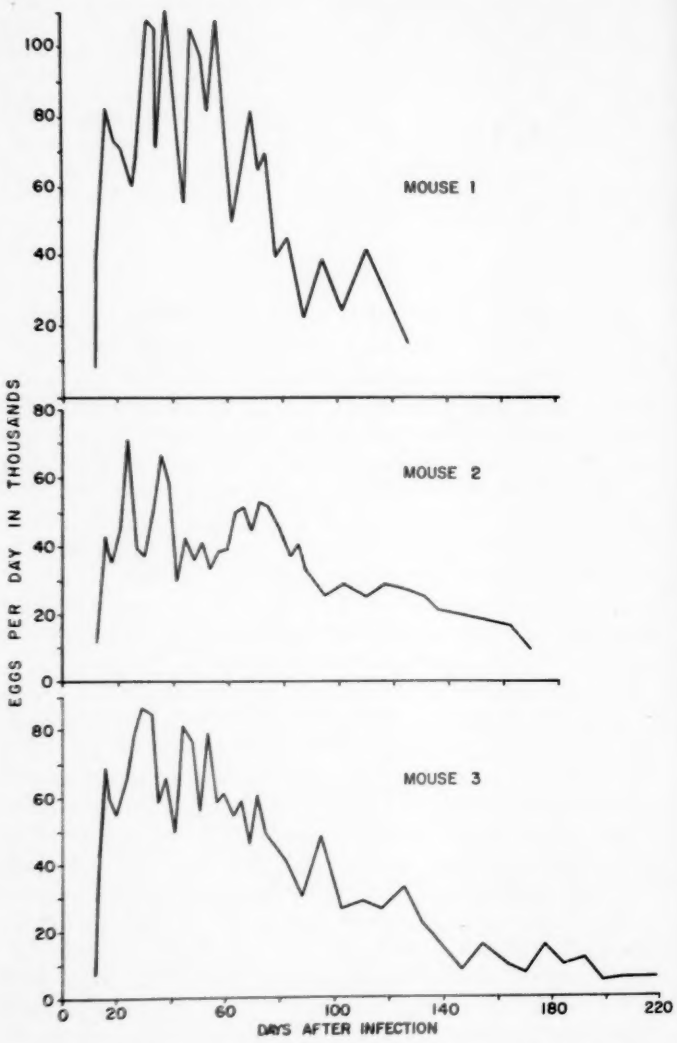

FIG. 1. The egg output of infections of *Nematospiroides dubius* in three mice from the day after eggs first appeared until the death of mice 1 and 2, and until the 220th day of infection in the case of Mouse 3.

In general the egg output rose rapidly from a low point the day after eggs first appeared to a peak value about a week later. The counts then fluctuated about a high level until about the 10th week, after which they decreased exponentially throughout the rest of the infection. In other words, during the period of decline the points were well fitted by a straight line on a semi-log plot. For example, in the case of the mouse followed for the longest time, the counts declined in accordance with the following exponential equation, where y represents the counts and x the days after infection.

$$y = \frac{136{,}000}{1.015^x}$$

Egg output per female worm in mice and rats. In the second series of experiments 2 mice and 2 rats were each given equal quantities of water estimated by dilution counts to contain 200 infective larvae. That this method of estimating allowed for some variation is obvious from the results but it seems clear that the major differences between the results for mice and those for rats cannot be attributed to this element of variability. The rats were given subcutaneous injections of cortisone acetate in amounts of 5 mg per 100 grams of body weight daily from the day of infection to the day when eggs appeared. Eggs first appeared in the mouse feces on the 11th day after infection, and in the rat feces on the 13th day. Counts were begun on the 11th day for the mice and were continued daily until the first peak had been passed. They were resumed on the 23rd day for a 5 day period, at the end of which the mice were killed. For the rats, counts were begun on the 14th day and continued through the 25th day, after which the rats were killed. The numbers of female worms recovered were 77 from Mouse 4, 155 from Mouse 5, 19 from Rat 1, and 27 from Rat 2. The large difference between the numbers of worms recovered from the two mice, which were supposedly given equal numbers of larvae, casts doubt on the adequacy of the dilution method used for estimating the numbers of larvae. It also raises a question regarding the comparability of results between rats and mice. Since the number of worms recovered from the rats is so much lower than the number recovered from either mouse, it seems safe to say that

the proportion of larvae developing in mice is very probably at least three times that in rats.

In order to make comparisons free from discrepancies based on the number of larvae given, the egg count for each day was divided by the number of female worms recovered from the particular animal. The resulting values are shown in Figure 2 in terms of eggs per day per female worm. Two additional mice (numbers 6 and 7) were each given approximately 100 larvae and counts made as before, except that the mice were killed on the 43rd day after infection. For all 6 animals in the second series of experiments the egg counts of the last 5 days before autopsy were used as a basis for calculating the average daily egg output per worm. The essential data are shown in Table 1. Analysis of variance of the differences within and between spe-

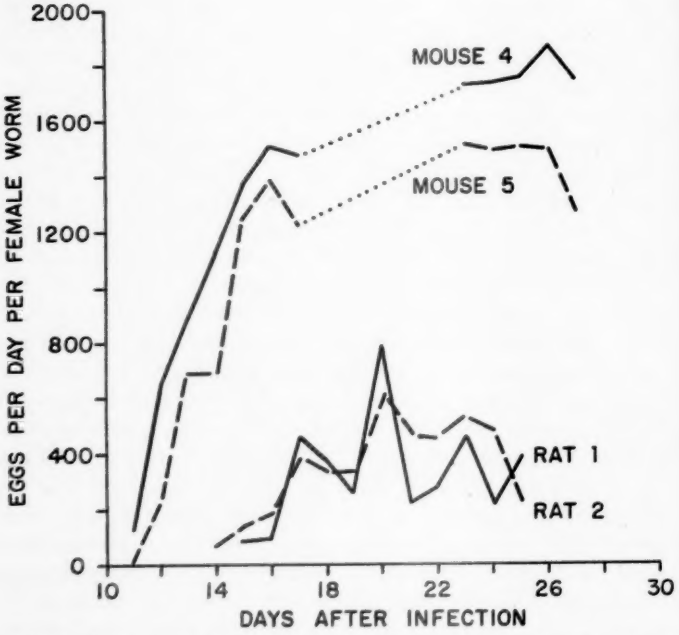

FIG. 2. Comparative egg production of *N. dubius* in mice and rats in terms of eggs per day per number of female worms present at autopsy on the day of the last count.

TABLE 1

Comparison in rats and mice of the number of worms recovered and the mean eggs per day per female worm for the last 5 days before autopsy

	Days from infection to autopsy	Worms recovered ♂	Worms recovered ♀	Eggs per day per female worm Mean	± S. E.
Rat 1	25	6	19	317	50
Rat 2	25	12	27	436	52
Mouse 4	27	42	77	1768	24
Mouse 5	27	90	155	1462	49
Mouse 6	43	27	63	1603	96
Mouse 7	43	59	90	1273	79

cies shows that the smallest mean egg output for mice is significantly different from the largest, but there are no other significant differences within species. The mean egg output per female worm is not related to the number of worms present. The differences between species are significant. Since the egg output for mice averaged about 4 times that for rats, the figures for rats were multiplied by 4 and the products were found to be not significantly different from the unmodified means for mice. In other words in addition to a larger proportion of worms developing in mice as compared to rats, each worm produces on the average about 4 times as many eggs in mice as in rats.

Discussion

The cycle of egg output of infections of *N. dubius* in mice is in general similar to that described for other intestinal nematode parasites. Immediately after eggs first appeared the counts rose rapidly to a high level. Although the subsequent trend is somewhat obscured by the day to day variability, a high level output appeared to be maintained from about the 3rd to the 8th or 10th week of infection. An interesting question is whether the decline in egg output which follows this period is the result of death or loss of worms, or is the result of decreasing egg production per worm. Evidence from other experiments (Cross, 1959) indicates that the worms produce some eggs up to the time of death since autopsies made immediately after eggs ceased to appear in the feces revealed no worms to be present. In the case of Mouse 3 no worms were found at autopsy, but the egg output from the 47th

to the 6th day before death was estimated to be 800 eggs per day. Even if only one worm had been present during this time, its indicated egg production would be considerably below that of worms in the mice listed in Table 1. These latter mice were killed at the peak of egg output and were estimated to be passing on the average 1526 eggs per day per female worm. By use of this latter figure and the average output between the 15th and the 35th day for Mice 1 and 2, we calculated that 66 and 36 female worms respectively should have been present. At death, however, after the egg output had fallen considerably, 49 and 32 female worms were actually present. These figures would indicate some loss of worms during this period of declining egg output.

The egg output of worms in the rat is of interest principally because this animal is an abnormal host of *N. dubius*. Ordinarily it is completely resistant to infection, but Cross (1959) has shown that when cortisone is administered during the first few days of infection, the resistance is overcome. The mechanism of this resistance appears to be a simple one. In the mouse the infective larvae penetrate to the submucosa of the intestine where they are surrounded by a mild inflammatory reaction. When they have developed nearly to the stage of sexual maturity, they return to the lumen of the intestine and soon begin to produce eggs. In the rat the principal difference is that the inflammatory reaction involves the formation of a connective tissue capsule which prevents the larvae from returning to the lumen. When cortisone is administered the formation of connective tissue is inhibited and the infection develops essentially as it does in the mouse. Although the initial resistance of the rat has thus apparently been completely overcome, there seem to be later phases which have not been affected. The infections in the rat generally terminate spontaneously after about 2 months, whereas in the mouse they usually last about 9 months. The data presented here show further evidence of immunity in that the egg output even at the peak is only ¼ that of the mouse. These later phases of immunity must be based on a different type of mechanism.

Summary

The egg output of infections in mice follows a pattern similar to that recorded for other nematodes. A rapid initial rise is fol-

lowed by a period of several weeks of relatively high output and then by an exponential decline. This decline apparently results, to a large extent, from loss of worms, although there may also be some reduction in the egg production per worm. When the initial resistance to infection of the rat is overcome by administration of cortisone, the proportion of larvae developing to maturity in rats is one third or less of that in mice. In rats the egg production per female worm during the high level period is only about one fourth that in mice. The earlier decline in egg production in rats as compared to mice is apparently due to earlier death of worms. Thus even though the initial resistance of rats can be overcome, there are significant later phases which must be based on a different immune mechanism.

REFERENCES AND NOTES

Baker, N. F., 1954. Trichostrongylidosis: The mouse as an experimental animal. *Proc. Am. Vet. Med. As.*, 91:185–191.

Cross, J. H., Jr. 1958. Natural resistance of the white rat to the mouse parasite *Nematospiroides dubius* Baylis, 1926 (Nematoda: Heligmosomidae). *Diss. Abs.* 19:6:1347–1348.

Cross, J. H., Jr. 1959. The natural resistance of the white rat to *Nematospiroides dubius* and the effect of cortisone on this resistance. In press.

Cross, J. H., Jr., Charlton Dawson, J. Allen Scott and Etta Mae MacDonald. 1958. Dilution egg counts of *Nematospiroides dubius* in mice and rats. *J. Parasit.* 44(supp.):32.

Ehrenford, F. A. 1954. The life cycle of *Nematospiroides dubius* Baylis (Nematoda:Heligmosomidae). *J. Parasit.* 40:480–481.

Scott, J. A. 1932. A pipette for the dilution counting of hookworm eggs. *Science* 76:170.

Spurlock, G. M. 1943. Observations on host-parasite relations between laboratory mice and *Nematospiroides dubius* Baylis. *J. Parasit.* 29:303–311.

Stoll, N. R. and Hausheer, W. C. 1926. Accuracy in the dilution egg counting method. *Am. J. Hyg.* 6(March supp.):80–133.

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STUDIES ON DENGUE FEVER*

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An oft-repeated question is "Has dengue fever ever occurred in the United States?" Older physicians in the South have frequently remarked about having seen this disease in their respective areas. The classic report by Griffitts (1) discusses in some detail the occurrence at Miami, Florida in 1934 of an epidemic clinically diagnosed as dengue. Unfortunately, the exact etiology of this epidemic is unknown. The diagnosis was based solely on symptomatology and epidemiology, as repeated attempts to isolate the infectious agent failed and the technics for serologic diagnosis were not yet developed. Since the development of such technics, this clinical entity has disappeared from this area, at least in its classic epidemic form. It is possible, however, that dengue infections occur or have occurred endemically or sporadically, as the known vector can be found locally. In addition, the endemic disease exists in the Caribbean, and the frequency of flights from this area and from Central and South America makes possible the importation of the agent.

For the past two years, this laboratory has been engaged in studies of dengue. One phase of the work has concerned the question of dengue infections in South Florida as reflected by hemagglutination inhibition antibodies.

Materials and Methods

A large number of sera from various age groups were available from an influenza vaccination study in 1957–58. Pre-immunization sera from children and from young and middle aged adults were selected for serologic studies. These sera had been stored at —20° C. In addition, specimens were selected at random from sera submitted to the virus diagnostic laboratory. These sera were from patients with a variety of clinical diagnoses, and all had been found to give negative results in the usual routine diagnostic serologic tests for virus infections.

The hemagglutination inhibition tests employed the technics of Sweet and Sabin (5) and of the Rockefeller Foundation Virus Laboratories (6). The Trinidad 1751 strain of dengue in the form of infected baby mouse brain suspension was used as antigen. To 0.25 ml aliquots of serum dilutions were added equal volumes of antigen containing 8 to 16 hemagglutinating units.

Serum-virus mixtures were stored overnight at 4° C, following which 0.5 ml of 0.25% goose cells was added to each tube. The final pH of the complete test was 6.5. Tests were read by observation of the pattern of RBC sedimentation after 1½ hours incubation at room temperature. The highest dilution of serum showing complete inhibition of hemagglutination was considered the end point, and serum titers were expressed as initial dilutions of serum (before addition of antigen and cells).

All sera were heat inactivated and were first screened utilizing the kaolin adsorption technic. Those which yielded positive results were re-tested following precipitation with acetone.

Results

Approximately 9% of the sera tested have been found to contain antibodies against the dengue virus. Table 1 illustrates these

TABLE 1

Hemagglutination inhibition tests with dengue virus

Group	Number tested	Positive HI Reactions (1:10 or greater)		Distribution of Titers			
		Number	Percent	1:10	1:20–1:40	1:80–1:160	1:320 or greater
Adults*	192	18	9.4	1	11	4	2
Children*	38	0	0	..	..	..	..
Random Diagnostic	19	2	10.5	0	0	1	1

* Preimmunization sera obtained in connection with an influenza vaccine study, 1957–58. HI test procedure employed acetone precipitated sera, goose cells and 8–16 units of antigen.

findings. Among the specimens taken in connection with the influenza vaccination study, 18 of 192 sera from adults were positive, while no reactors have been found among the small group of children tested to date. Of the 19 randomly selected diagnostic sera tested, paired specimens from two patients were found to be positive. In neither instance was an antibody rise demonstrable. Examination of the antibody titer distribution shows that 40% of the reactors had titers of 1:80 or higher. Repeated testing of individual sera indicated excellent reproducibility of end-points. A small group of sera, including both positives and negatives, was forwarded to Dr. Benjamin Sweet of the University of Maryland for confirmation of our findings. His results were almost identical with those obtained in this laboratory.

A number of positive and negative sera, with respect to HI

antibodies, were subjected to neutralization tests with dengue virus. The tests employed undiluted heat-inactivated serum and serial dilutions of virus in 10% fresh unheated rabbit, calf or guinea pig serum. The neutralization test results correlated perfectly with the *in vitro* serology, in that all sera with HI antibodies gave positive neutralization results while those lacking HI antibodies were completely devoid of neutralizing capacity.

Table 2 indicates the individual HI titers and neutralization indices obtained, and summarizes some of the pertinent background information on individuals with positive dengue HI tests. Race is not indicated, but all were Caucasians except for H.R., the fourth person listed. In some instances, antibodies may have arisen as a result of yellow fever immunization or of infection acquired abroad. It is to be noted, however, that there are several individuals who have never left the continental United States

TABLE 2

Table 2. Background information on persons with positive dengue HI tests.

Identity	Dengue Serology		Age	Sex	Birthplace	Dates of Residence in S. Fla.	Mil. Svc.	Yellow Fever Vaccine	Travel Abroad
	HI Titer	Neut. Index							
R.R.*	1:40	354	41	F	Florida	Life	No	No	None
C.L.D.**	1:40	575	53	M	N.Carolina	23-26;37-59	No	No	None
L.A.S.	1:80	8	45	M	Georgia	ND	No	No	None
H.R.	1:160	ND	44	M	Georgia	49-59	No	No	None
E.C.A.	1:40	19	38	M	ND	ND	ND	ND	ND
M.G.	1:20	ND	43	F	Cuba	38-59	No	No	None
M.P.	1:40	ND	20	F	PuertoRico	45-59	No	No	None
R.L.S.	1:10	ND	48	M	Cuba	46-59	No	No	Cuba, occasional
J.C.	1:320	ND	57	M	Spain	18-59	No	No	Cuba, brief,1946
Y.E.S.	1:20	ND	42	M	Florida	Life	No	No	Puerto Rico 1950
M.A.C.	1:320	ND	24	F	Cuba	ND	No	No	Cuba semi-annual
H.C.K.	1:80	ND	38	F	PuertoRico	41-59	No	No	Puerto Rico yrly
F.L.McG.	1:20	ND	46	M	Illinois	ND	Yes	No	Europe, Bermuda, Africa,'41-45.
P.V.S.	1:40	ND	31	M	Florida	Life	Yes	No	Trinidad, Cuba
M.F.	1:20	48	33	M	Florida	25-59	Yes	No	Europe, '44-46.
R.D.F.	1:40	ND	34	M	Indiana	57-59	Yes	No	British Guiana
S.R.F.	1:320	ND	50	M	New York	22-59	Yes	No	Canada, 1954.
W.F.G.	1:80	631	37	M	Tennessee	ND	No	Yes '57	Panama,Honolulu
G.G.H.	1:160	ND	48	M	Florida	Life	ND	Yes '46	South America
J.E.H.	1:40	ND	35	M	Georgia	ND	Yes	Yes	Africa, England, Korea, Japan, So. America

ND = No Data
* Parents alleged to have had dengue in 1925-26.
** Reports having had "dengue fever" in 1925-26.

nor received yellow fever vaccine. Of special interest are the first two persons listed. R.R. has indicated that her parents both had dengue fever, and C.L.D. reports having had the disease himself. In both instances, the illnesses are alleged to have occurred in 1925–26.

In October and November of 1958, this laboratory detected serologic evidence of St. Louis encephalitis (SLE) in 3 patients residing in South Florida. This was the first such evidence noted since establishment of the laboratory in 1955. In the fall of 1958, there also appears to have been an increase in the population of *Culex quinquefasciatus* in this area, as reported by County and State entomologists. The finding of SLE was not surprising, as serologic evidence of such infections in birds, animals and man in Jamaica, W.I., were obtained by Sigel and Grant in 1954 (unpublished). These findings were confirmed by the Trinidal Regional Virus Laboratory (4). In addition, Sanders, Blumberg and Haymaker (5) reported in 1953 the isolation of SLE virus from a fatal case occurring in South Florida.

Since dengue and St. Louis encephalitis viruses are antigenically related, these findings raised a question as to the specificity of the HI results with the dengue antigen. The sera of 2 of the dengue reactors and convalescent sera of the 3 St. Louis encephalitis patients were therefore re-tested with dengue and SLE antigens by HI and neutralization tests. Results are shown in Table 3. There appeared to be little if any cross-reactivity in the

TABLE 3

Reciprocal serologic testing of sera demonstrated to have Dengue or SLE antibodies

		Tests with Dengue		Tests with SLE	
Group	Identity	HI titer	Neutralization index	CF titer	Neutralization index
Dengue Reactors	R.R.	1:40	354	ND	Neg.
	L.A.S.	1:80	10	ND	Neg.
SLE Reactors*	M.B.	<1:10	Neg.	1:16	68
	E.B.	<1:10	Neg.	1:16	10
	A.V.	<1:10	Neg.	1:16	32

ND = No Data.
* Convalescent sera; acute sera = neg. CF for SLE.

various tests, and results were quite clean cut. Although the neutralization indices with SLE were rather low, they—as well as the CF titers—were of the same order as found by one of the authors in sera tested during a St. Louis encephalitis epidemic in Texas in 1954 (6).

Discussion

It is well known that when double or multiple infections with members of the Group B arthropod-borne viruses occur in an individual, antibodies with wide overlaps with other members of the group are produced and interpretation of serologic tests may be difficult or virtually impossible. As contrasted with many parts of the world where several such viruses may be endemic in a single locale and superinfections may be common, the area of South Florida may be an ideal place for studies of homotypic antibody responses to certain of these agents and for determination of the duration of demonstrable antibodies. The relatively high degree of sanitation, window screening and better mosquito control would be expected to be associated with a low incidence of infections with the Group B arthropod-borne viruses in Florida. That such may be the case is illustrated by the sharp distinctions in the serologic tests with SLE and dengue shown in Table 3. Heterotypic responses, however, would also be encountered, especially among émigrés from other parts of the world. A recent instance of this occurred in a Puerto Rican now residing in Miami, who presented a clinical picture compatible with SLE and had antibody titers to both SLE and dengue as measured by HI, CF and neutralization tests.

It is apparent that infections with an agent or agents antigenically closely related to dengue virus, if not actually dengue, have occurred or are occurring in South Florida. The same is true of St. Louis encephalitis. In addition, recent collaborative studies by Pennington and Pond of the National Institutes of Health and Ehrenkranz of this University (2) suggest the presence in this area of at least one other member of the B group, as one native resident has been found to possess antibodies to Ilheus virus.

Further investigations of the exact nature of the agents involved are in order, and have been instigated by this group. With collaboration of State and County epidemiologists, pools of mos-

quitoes are being collected, and virus isolation from these will be attempted. Collection of sera from wild and domestic animals and from birds has begun. These sera, plus those submitted to the virus diagnostic laboratory, will be tested for serologic evidence of infections with dengue and with other members of Group B of the arthropod-borne viruses. With such an approach, it is hoped to clarify the question of whether infections with dengue virus occur in South Florida.

BIBLIOGRAPHY AND NOTES

1. Griffits, T. H. D. Dengue in Florida, 1934, and its significance. *Jour. Flor. Med. Asso.* 21: 395–397. 1935.
2. Pond, W., R. Pennington and J. Ehrenkranz. Personal communication.
3. Sanders, M., A. Blumberg, and W. Haymaker. *Southern Med. Jour.* 46: 606–608. 1953.
4. Sullivan, Th. D., J. V. Irons and M. M. Sigel. St. Louis Encephalitis in Hidalgo county, Texas. Laboratory aspects. *Publ. Health Rep.* 72: 526–530. 1957.
5. Sweet, B. H. and A. Sabin. Properties and antigenic relationships of hemagglutinins associated with the dengue viruses. *Jour. Imm.* 73: 363–373. 1954.
6. Technical Manual. "Hemagglutination with arthropod-borne viruses" Rockefeller Foundation Virus Laboratories, New York. 1955.
7. Trinidad Regional Virus Laboratory, Port-of-Spain, Trinidad. W. I. Personal communication.

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A BACTERIOLOGICAL TECHNIQUE FOR THE ISOLATION OF MARINE DIATOMS

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These observations were noted during bacteriological studies of marine environments.

Samples of estuarine waters, sediments, and plankton tows were diluted quantitatively with sterile, aged sea water. Aliquots of each dilution, 50–500 ml., were filtered through 'millipore filter' membranes (Millipore Filter Corp., Bedford, Mass.). Filters were placed, right side-up, on the surface of petri-plates which contained a medium of the following composition: aged sea water to volume; Difco agar, 1.0 per cent; sodium acetate, 0.01 per cent; and yeast extract, trypticase, and peptone, 0.05 per cent each. The final pH before autoclaving at 15 lb. for 15 min. was pH 7.6–7.8. Cultures were incubated under a battery of fluorescent lights at 22° C.–25° C. for 1–3 wk. Using a binocular microscope with a magnification of 5×–10×, typical diatom tracts were observed as a network surrounding the filter and extending outward along the surface of the agar. Bacterial and algal colonies were confined to the edge or surface of the filter. Quite often, samples of sediments and plankton tows would contain agar-digesting bacteria which would liquefy the agar along the edge of the filter and inhibit the typical outward migration of the diatoms.

A fine platinum wire needle was used to cut small pieces of agar containing cells from the extreme edge of the diatom network. The agar blocks were transferred to sterile plates of the above composition and were incubated until diatom tracts were observed again. Usually best results were obtained by placing each agar block with the cell side in contact with the medium. In this manner, several serial transfers were made in order to insure culture purity. Sterility tests were made in test tubes of the same medium minus the agar.

Using this technique, several species of *Nitzschia* and *Navicula* were isolated and maintained in pure culture. Other genera were obtained on occasion but could not be successfully trans-

ferred more than 1 or 2 times in pure culture. This could be attributed to a number of causes including lack of essential nutrients. It is suggested that variation in the chemical composition of the isolation medium may facilitate the isolation of the more fastidious diatoms.

Other investigators have used the migration characteristic of microorganisms for cultures of amebae (1,2) and Myxomycetes (3). Provasoli and Pintner (4) described the rapid growth of the filaments of *Phormidium persicinum*, an auxotrophic marine red-pigmented blue-green alga, as a means of eliminating bacterial growth. Other workers (5) used antibiotics in their initial isolation medium. Watson (6) used the agar block technique for the isolation of *Labyrinthula*, the causative agent of wasting disease of *Zostera marina* (ell-grass). In most instances, isolations depended on the migratory ability of the organism.

REFERENCES AND NOTES

1. Beijerinck, M. W.: Amöbenkultur auf festen Substraten. *Centralbl. Bakt.*, 21: 101–102, 1897.
2. Oehler, R.: Amöbenzucht auf reinem Boden. *Arch. Protistenk.*, 37: 175–190, 1916.
3. Cohen, A. L.: Nutrition of the Myxomycetes. I. Pure culture and two-membered culture of Myxomycete Plasmodia. *Bot. Gazette*, 101: 243–275, 1939.
4. Provasoli, L. and Pintner, I. J.: Cultural characteristics of *Phormidium persicinum* an auxotrophic marine red-pigmented blue-green alga. *Rapports et Communication du VIII Congres Inter. de Botanique., Sect. 17*, pp. 39–40, 1954.
5. Hutner, S. H. and Provasoli, L.: A pigmented marine diatom requiring vitamin B_{12} and uracil. *Phycological News Bull.*, 18: 7–8, 1953.
6. Watson, S. W.: Studies on *Labyrinthula*. Master's thesis, Univ. of Wash., 1951.

[a] From the Department of Preventive Medicine and Public Health, University of Texas Medical Branch, Galveston. Paper accepted Aug. 10, 1959.

STUDIES ON A CULTURE MEDIUM FOR THE IDENTIFICATION OF PATHOGENIC SYSTEMIC FUNGI

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Little progress has been made toward formulating a medium which is superior to that used by Sabouraud in 1893. Many of the media commonly used today are selective for the cultivation of certain fungi and the majority of experimental observations have been concerned with specific growth requirements such as vitamins (1, 2, 5, 6, 8, 12, 13), nitrogen sources (9, 11, 14, 15), carbon sources (7, 10, 16), trace elements (4), pH, or environment. These studies have, for the most part, been limited to the saprophytic fungi or the Dermatophytes.

In recent years investigators have become interested in the pathogenic systemic fungi. In 1947 Campbell (2) was able to demonstrate the dimorphism of *H. capsulatum* by the use of a special blood-cysteine medium. Other workers have studied the factors which influence the growth of organisms such as *Nocardia*, *C. neoformans*, *B. dermatitidis* (15), *S. schenckii*, and *C. immitis* (3), but thus far these studies have revealed that apparently there is no single nutrient element or environment which will support maximum growth of all these fungi. This study was undertaken to investigate the efficacy of a single medium containing all of the elements known to be necessary for isolation and identification of the pathogenic systemic fungi.

Materials and Methods

The Basal medium consisted of Bacto-Soytone (Difco) in a final concentration of one per cent and solidified by the use of 1.5% Bacto-agar (Difco). Preliminary studies revealed that the rate of growth, sporulation and pigmentation of the test fungi were comparable with 0.5%, 1.0%, 1.5% or 2.0% Bacto-Soytone. Experiments employing a soybean infusion prepared from soybean flour showed considerable variation in the rate of growth of fungi depending upon the lot of flour. Results with Bacto-Soytone were consistently reproducible and superior to those obtained with the soybean infusion. The effects of supplementary nitrogen sources in the form of Protone (Difco), Tryptone (Difco) and Tryptose (Difco) were studied.

In this study only those carbohydrates known to be utilized by a majority of the fungi were tested. These carbohydrates: maltose (Pfansteihl), dextrose (Difco), cerelose (Corn Product Sales Co.), trehalose (Difco) and sucrose (Difco) were added singly and in various combinations to a final concentration of from 1 to 5% total carbohydrate.

A stock solution of trace elements containing 10^{-3} molar equivalents per 100 ml. of each of the following: cobalt ($CoSO_4 \cdot H_2O$), iron ($FeSO_4 \cdot 7H_2O$), calcium ($CaCl_2$), copper ($CuSO_4 \cdot 5H_2O$), magnesium ($MgCl_2$) manganese ($MnSO_4$), and zinc ($ZnSO_4 \cdot 7H_2O$) was prepared and 0.1 ml was added per liter of medium to produce a final concentration of 10^{-7} mol. equivalents. The medium was autoclaved for 10 minutes at 10 lbs. pressure and the resulting pH after autocloving was 5.0–5.5. Petri plates were poured at least ⅓ full, and preferably ½ full.

Test organisms included *Cryptococcus neoformans* (strains I, II), *Candida (Monilia) albicans*, *Nocardia asteroides*, *Coccidioides immitis*, *Blastomyces dermatitidis*, *Histoplasma capsulatum* and *Trichophyton megnini*. These fungi were originally obtained from the Communicable Disease Center, Chamblee, Georgia, and were maintained on Sabouraud's dextrose agar at Baylor University College of Medicine.

In preparing inoculum, colonies of *C. albicans*, *C. neoformans* and *N. asteroides* were harvested after 7 days incubation at 37° C by washing the slant with sterile distilled water. Residual medium was removed by centrifugating and washing at least 3 times with sterile water. The number of cells employed in test was approximately 5×10^8 cells per ml as determined by Neubar chamber method. Due to spontaneous clumping of *N. asteroides* direct counts were impractical and it was diluted so that the turbidity was grossly relative to a McFarland No. 1 Standard. Plates of the experimental medium were inoculated with a 2 mm. bacteriological loop and incubated at 37° C.

Cultures of *B. dermatitidis*, *H. capsulatum*, *C. immitis* and *T. megnini* were grown in Sabouraud's dextrose broth for 7 days at 25° C. A single colony approximately 2 mm. in diameter was picked, washed, and transferred to appropriate media. Cultures were incubated at 25° C.

Examinations of cultures were made at 2, 4, 6, 8 and 10 day intervals and growth recorded both qualitatively and quantitatively. All results recorded were an average of triplicate series. Qualitatively, growth comparisons were made by measuring the diameter of the colonies with no allowance for height. Quantitatively, growth was estimated by the use of dry weight and micro-Kjeldahl protein determination on the entire colony at the end of 10 days growth. The latter procedure was feasible only with *N. asteroides*, *C. albicans* and *C. neoformans* (I, II) as the colonies could be readily freed from the agar and washed. It is possible that the absolute protein values of *C. neoformans* may be low, due to the loss of soluble capsular material during the repeated washings.

Results

The final medium was derived after many experiments utilizing different concentrations and/or combinations of the follow-

ing carbohydrates (dextrose, maltose, trehalose, cerelose, sucrose) and peptones (tryptone, tryptose, protone, soytone). Based upon observations of the amount of growth obtained, it was found that a soytone base with added protone and a mixture of maltose and glucose gave superior results over any other combination of nutrients. In testing the various carbohydrates con-

TABLE 1

Quantitative comparisons of growth of the yeast-like fungi following 10 days incubation at 37° C.

Test Organism	Medium used	Dry weight in MGM	Protein in MGM	Increase obtained in percent	
				Dry weight	Protein
N. asteroides	Sabouraud's	40.0	32.8	40.2	39.9
	GMS	56.1	45.9	...	...
C. albicans	Sabouraud's	39.6	34.5	95.0	79.1
	GMS	77.2	61.9	...	...
C. neoformans I	Sabouraud's	25.3	24.1	76.6	74.7
	GMS	44.7	42.1	...	...
C. neoformans II	Sabouraud's	27.1	26.4	21.0	10.7
	GMS	32.8	29.2	...	...

tained in the medium, it was found that the addition of Seitz filtered sugars had no advantage over the medium in which the carbohydrates were autoclaved at 10 lbs. pressure for 10 minutes. The addition of trace elements to this medium had no visible effect upon the yeast type of colonies although pigmentation was increased in *N. asteroides* and *T. megnini*. The optional experimental medium (GMS) consisted of 10 gms Bacto-Soytone (Difco), 15 gms Bacto-Agar (Difco), 20 gms Maltose (Pfansteihl), 30 gms Dextrose (Difco), 2.0 gms Protone (Difco) and 0.1 ml stock solution of trace elements per liter.

As shown in Table 1, the dry weight and protein content of the colonies of *N. asteroides*, *C. albicans* and *C. neoformans* (I, II) on GMS was much greater at the end of 10 days than that obtained on the Sabouraud's dextrose agar for a comparable period of time.

Table 2 illustrates the comparative colony diameters of *C. immitis*, *H. capsulatum*, *T. megnini* and *B. dermatitidis* on Littman's oxgall agar (Difco), Sabouraud's dextrose agar (Difco),

TABLE 2

Quantitative comparison of colony size of filamentous type of fungi after 21 days incubation at room temperature.

Test organism	Test medium		
	Littman Oxgall	Sabouraud's	GMS
*C. immitis**	8.5 cm	8.7 cm	9.2 cm
H. capsulatum	2.6 cm	4.0 cm	5.8 cm
B. dermatitidis	5.6 cm	6.5 cm	8.7 cm
T. megnini	2.1 cm	6.2 cm	6.8 cm

* Growth recorded at end of 14 days incubation, due to the luxuriant growth on GMS.

and GMS following 21 days incubation at room temperature. Not only did these four filamentous fungi attain a greater size on GMS but they also began sporulation within the first week, whereas this stage was not evident on Sabouraud's until after the tenth day. *T. megnini*, on the GMS medium, produced an abundance of microconida and pigmentation was very intense. Colonies on Littman's agar were consistently smaller when compared to Sabouraud's and GMS. It should be noted that Littman's agar is a selective medium employed chiefly for the primary isolation of fungi and thus such comparisons may not be valid. Of all the fungi tested, none showed any abnormalities of colony characteristics which is so often the case in various selective media.

Discussion

In a detailed comparative analysis of the amino acids, vitamins and trace elements contained in a particular nutrient base,* the most obvious difference was in the amount of the individual components. For example, Bacto-Neopeptone (base for Sabouraud's agar), differs mainly from Bacto-Soytone and Bacto-Protone in the amount of individual amino acids, vitamins and trace elements contained.

For the observations made in this study, it would appear that the relative amounts of certain utilizable nutrients could account for the differences in the growth rates obtained with the experimental medium.

The addition of trace elements is known to have a profound effect on reproduction, pigmentation, and metabolism of fungi

(4), but has been studied chiefly in the filamentous type of fungi. By the incorporation of trace elements into the GMS, it was observed that pigmentation and sporulation were greatly enhanced but the effect on growth was not significant. The pigments of *N. asteroides* and *T. megnini* were intensified in GMS when contrasted with Sabouraud's and the sporulation of *B. dermatitidis*, *C. immitis*, and *H. capsulatum* appeared earlier on the GMS.

From the results obtained, there was apparently no one single factor involved to account for the stimulated growth and reproduction observed. From our observations it appeared that the quantity available of an essential vitamin, nitrogen source, carbon source, or trace element may hold the key to both growth and reproduction, rather than some unknown element.

Summary

A non-selective medium containing soytone, protone, maltose, glucose, and trace elements was found to stimulate growth, sporulation and pigmentation of all fungi tested. This medium does not affect the general colonial characteristics or any other property used for identification.

REFERENCES AND NOTES

1. Burkholder, P. R., McVeigh, I., and Moyer, D. Studies on some growth factors of yeast. *J. Bact.*, 48: 385–391, 1944.
2. Campbell, C. C. Reverting *Histoplasma capsulatum* to the yeast phase. *J. Bact.*, 54: 263–272, 1947.
3. Converse, J. L. Growth of spherules of *C. immitis* in a chemically defined liquid medium. *Proc. Soc. Exp. Biol. & Med.*, 90: 709–711, 1955.
4. Foster, J. W. "Chemical Activities of Fungi." Academic Press Inc., New York, N.Y.
5. Georg, L. K. The relation of nutrition to the growth and morphology of *Trichophyton violaceum*. 1. The vitamin and amino acid requirements. *Mycologia*, 43: 297–313, 1951.
6. Halliday, W. J. and McCoy, E. Biotin as a growth requirement for *Blastomyces dermatitidis*. *J. Bact.*, 70: 464–469, 1955.
7. Horr, W. H. Utilization of galactose by *Aspergillis niger* and *Penicillum glaucum*. *Plant Physiol.*, 11: 81–89, 1936.
8. Lilly, V. G., and Barnett, H. L. The influence of concentration of nutrients, thiamine and biotin upon growth and formation of perithecia and ascospores by Chatonium convolutum. *Mycologia*, 41: 186–195, 1949.
9. Lurie, H. I. Sporotrichium species: their nitrogen metabolism. *Mycologia*, 43: 117–124, 1951.
10. McClung, N. M. Utilization of carbon compounds by Nocardia species. *J. Bact.*, 68: 231–236, 1954.

11. McVickar, D. L. Factors important for the growth of *Histoplasma capsulatum* in the yeast phase on peptone media. *J. Bact.*, 62: 137–143, 1951.
12. Moat, A. G. and Emmonds, E. K. Thiamine acid nutrition of yeast in relationship to biotin deficiency. *J. Bact.*, 68: 687–690, 1954.
13. Reid, J. D. The influence of the vitamin B complex and the growth of *Torulopsis (Cryptococcus) neoformans* on a synthetic media. *J. Bact.*, 58: 777–781, 1959.
14. Salvin, B. S. Cysteine and related compounds in the growth of the yeast-like phase of *Histoplasma capsulatum*. *J. Infect. Dis.*, 84: 275–283, 1949.
15. Scherr, G. H. and Weaver, R. H. The dimorphism phenomenon in yeasts. *Bact. Rev.*, 17: 51–85, 1953.
16. Schultz, A. S., McManus, D. K. and Powper, S. Amino acids as carbon sources for the growth of yeasts. *Arch. Biochem.*, 22: 412–418, 1949.

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* This information was furnished by the various biological houses marketing each of the different media mentioned under *Materials and Methods*.

EVIDENCE FOR LIMITED ACTH SECRETION BY PITUITARY AUTOGRAFTS IN THE RENAL CAPSULE OF THE RAT[a]

RICHARD F. TIMMER, ALBERT E. SANDERS AND EDWARD G. RENNELS[b]

We have been studying the cytology and functional capacity of the pituitary gland of the rat following its autotransplantation to the renal capsule. We confirmed the earlier reports of Desclin (1950) and Everett (1954) that such grafts are capable of luteotrophin secretion and have assigned this secretory activity to the orange type of acidophile as seen in Azan preparations (Sanders & Rennels, 1959). In grafts that have been in the kidney for 2½ to 4 months, we find no evidence for spontaneous secretion of appreciable amounts of any of the other anterior lobe hormones. The present report relates our findings in regard to the capacity of such pituitary autografts to secrete ACTH in response to stress, as measured by the adrenal ascorbic acid depletion test.

Materials and Methods

The animals used in this study were rats of the Holtzman strain. Although both males and females were studied, they are combined for purposes of analysis since no marked sex difference was noted in the adrenal responses. Operative procedures were carried out when the animals were 40 to 47 days of age and experiments were terminated at periods of 2½ to 4 months following the operation. The animals fall into the following groups: (1) intact controls, (2) hypophysectomized controls, (3) hypophysectomized animals bearing autografts, and (4) incompletely hypophysectomized animals. Hypophysectomy was performed by the usual parapharyngeal approach and when the gland was transplanted this was accomplished within a brief period of time. The animals considered here are but a few of a much larger series of hypophysectomized and autograft-bearing rats that have been studied. Except for the incompletely hypophysectomized group listed in Table 1, in all cases the completeness of the original hypophysectomy as well as the viability of the graft was positively established by a study of body growth, hair maturation and a careful analysis of all organs known to reflect pituitary function (Sanders & Rennels, 1959). One hour prior to autopsy the left adrenal gland was removed surgically, cleaned, weighed and placed in 6% trichloroacetic acid for ascorbic acid analysis. Such a procedure constitutes an acceptable method of discharging ACTH from an intact animal's pituitary (Sayers *et al.*, 1948) and was used as a test of the ability of the pituitary grafts to

release ACTH. The right gland was similarly treated at autopsy and both glands were analyzed for ascorbic acid content by the method of Roe & Kuether (1943). In the case of the smaller glands of the hypophysectomized and autograft-bearing rats, the volume of acid used for extraction was appropriately reduced in order to increase the accuracy of the colorimetric determinations. The readings were made on a Coleman Jr. colorimeter.

Results and Discussion

The results are given in Table 1 in which average body weight, adrenal weight and percent depletion of adrenal ascorbic acid ($\frac{L\text{-}R}{L} \times 100$) resulting from surgical stress are listed. The pituitary autograft completely fails to maintain the adrenal weight above that of the hypophysectomized controls. In fact, on a relative to body weight basis, adrenal glands of the hypophysectomized animals are slightly heavier than those of the autograft-bearing animals. This agrees with other reports which indicate the inability of a pituitary graft to maintain adrenal weight (Harris, 1955). It should be noted also that the adrenal weight is rather well maintained in those animals in which a pituitary fragment remains in the sella.

In view of the failure of the pituitary graft to maintain adrenal weight, it is significant that the stress of surgery in these animals is capable of effecting a depletion of adrenal ascorbic acid which, percentagewise, approaches that seen in intact animals or animals incompletely hypophysectomized. In contrast, it is seen, as expected, that no change occurs in adrenal ascorbic acid of totally hypophysectomized animals in response to stress. Furthermore, it should be noted that the concentration of adre-

TABLE 1

Adrenal response to surgical stress in rats

Group	No.	Body wt. g.	Adrenal wt. mg./100 g. body wt.	Ascorbic acid (percent depletion)
Intact Controls	11	347	17.8 ± 1.9*	52.4
Hypex. Controls	19	142	8.1 ± .3	— 1.1
Hypex. + Autografts	14	182	6.8 ± .3	33.7
Incomplete Hypex.	6	337	12.0 ± .5	38.8

* Standard Error.

nal ascorbic acid declines after hypophysectomy, to levels of about 200 mg./100 g. adrenal tissue (185 ± 10). This is similar to the values that have been reported for the atrophic glands of hypophysectomized animals (Tyslowitz, 1943; Covian, 1949). The resting level found in the adrenal gland of the pituitary autograft-bearing animals (202 ± 14) is only slightly above this value, but unlike that of the hypophysectomized animal it is depleted following the stress of surgery. It follows that some factor capable of depleting adrenal ascorbic acid is released as a result of surgical stress and, until proved otherwise, it seems reasonable to assume that this factor is ACTH. Unfortunately, the meaning of this depletion in terms of the quantity of ACTH involved cannot be determined until we have more information concerning the sensitivity to ACTH of the atrophic adrenals at such long periods after hypophysectomy.

Other workers have reported that pituitary grafts are capable of releasing limited amounts of ACTH (Cheng *et al.*, 1949; McDermott *et al.*, 1950; Fortier, 1952). Previous reports have dealt with grafts in sites other than the renal capsule and the number of animals studied has often been rather limited. Fortier (1952) claimed that grafts in the anterior chamber of the eye would respond to systemic stressors but failed to respond to what he termed neurotropic stimuli. The grafts which he studied were all placed in the anterior chamber and the eosinopenic response to stress served as the index of ACTH discharge. Using the adrenal ascorbic acid response, Cheng *et al.* (1949) found that pituitary grafts in the eye or spleen of the rat were capable of releasing some ACTH in response to injection of histamine.

Our results offer conclusive proof that pituitary autografts in the renal capsule are capable of effecting a marked depletion of adrenal ascorbic acid in response to surgical stress. Such results must be given consideration in any hypothesis which attempts to explain the control mechanism for ACTH secretion. If hypothalamic neurohumors are essential to ACTH release then we must assume that such mediators act systematically on the cells of the pituitary graft. Apparently, a spontaneous secretion of ACTH from the pituitary autograft in the kidney does not occur at a level capable of affecting adrenal weight. In spite of this, it is clear that a marked adrenal ascorbic acid depletion can occur in response to surgical stress. It is not yet known, however, if

this change in ascorbic acid is accompanied by a change in the rate of corticosteroid secretion. More study of such aspects is needed before a final analysis of the control mechanism for ACTH secretion can be made.

Summary

A limited capacity of pituitary autografts to release ACTH has been demonstrated by the ability of such grafts to effect adrenal ascorbic acid depletion following surgical stress. The animals studied were rats of the Holtzman strain in which the pituitary gland was autotransplanted to a renal capsule. In experiments lasting for 2½ to 4 months such grafts completely failed to maintain the weight of the adrenal glands. In spite of this evidence of inactivity, the grafts are capable of effecting a depletion of adrenal ascorbic acid which approaches that seen in intact animals or animals incompletely hypophysectomized. These results indicate a limited but definite capacity of pituitary autografts to secrete ACTH.

REFERENCES AND NOTES

Cheng, C. P., G. Sayers, L. S. Goodman, and C. A. Swinyard: Discharge of Adrenocorticotrophic Hormone from Transplanted Pituitary Tissue. *Am. J. Physiol.*, 159: 426–432, 1949.

Covian, M. R.: Variaciones del Ácido Ascórbico Suprarrenal en la Rata Blanca Hipofisectomizada. *Rev. Soc. argen. biol.*, 25: 26–30, 1949.

Desclin, L.: A Propos du Mechanism D'action des Oestrogènes sur le Lobe Anteriéur le Hypophyse Chez le Rat. *Ann. Endocrinol.*, 11: 656–659, 1950.

Everett, J. W.: Luteotrophic Function of Autografts of the Rat Hypophysis. *Endocrinology*, 54: 685–690, 1954.

Fortier, C.: Studies on the Control of ACTH Release by Means of Hypophyseal Transplants. *Ciba Foundation Colloquia on Endocrinology*, 4: 124–136, 1952.

Harris, G. W.: *Neural Control of the Pituitary Gland*. Edward Arnold, Ltd., London, 1955.

McDermott, W. V., E. G. Fry, J. R. Brobeck, and C. N. H. Long: Release of Adrenocorticotrophic Hormone by Direct Application of Epinephrine to Pituitary Grafts. *Proc. Soc. Exp. Biol. & Med.*, 73: 609–610, 1950.

Roe, J. H. and C. A. Kuether: The Determination of Ascorbic Acid in Whole Blood and Urine Through the 2,4-dinitrophenylhydrazine Derivative of Dehydroascorbic Acid. *J. Biol. Chem.*, 147: 399–407, 1943.

Sanders, A. E. and E. G. Rennels: Evidence on the Cellular Source of Luteotrophin Derived from a Study of Rat Pituitary Autografts. *Z. Zellforsch.*, 49: 263–274, 1959.

Sayers, M. A., G. Sayers, and L. A. Woodbury: The Assay of Adrenocorticotrophic Hormone by the Adrenal Ascorbic Acid-Depletion Method. *Endocrinology*, 42: 379–393, 1948.

Tyslowitz, R.: Effect of Hypophysectomy on the Concentration of Ascorbic Acid in the Adrenals of the Rat. *Endocrinology*, 32: 103–108, 1943.

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THE LETHAL EFFECT OF CORTISONE ON MOUSE EMBRYOS WITH SPONTANEOUS CLEFT LIP-CLEFT PALATE[a]

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Numerous experiments with vitamin deficiencies or excesses, hormone excesses, and various other deleterious treatments administered to the pregnant female, have shown that congenital malformations can arise in mammalian embryos as a consequence of adverse environmental influences (3, 8). Disease can also cause defects as shown by the well documented correlation of maternal rubella with congenital anomalies. Thus, one would expect that the great improvements in nutrition, sanitation and treatment of diseases achieved in recent years would have resulted in a marked decrease in the incidence of congenital malformations. However, their frequency has not only failed to decrease, but has actually increased slightly (1, 14). Warkany (14) has suggested this increase may be due to improved diagnosis. Nevertheless, the absence of a marked decrease appears to contradict the current concept of a strong environmental factor in the etiology of congenital malformations.

One possible explanation for this persistent high frequency of congenital anomalies is that some adverse environmental agents selectively destroy malformed embryos and that the removal of these agents allows such defective embryos to reach maturity. Thus, the babies saved from malformation through an improvement of their environment might be counterbalanced by a group of defective embryos reaching maturity that otherwise (in the unimproved environment) would have perished. It is not yet possible to test this hypothesis with data from human material. However, a relevant situation has arisen in an experiment with mice involving a deleterious environmental agent. Cortisone, when administered in massive doses to pregnant female mice, can cause cleft palate (4). The interaction of this teratogen with the spontaneously occurring cleft lip-cleft palate of the A/Jax strain of mice affects the frequency of the spontaneous anomaly in a manner that gives experimental support to the hypothesis

that an improved environment can result in the birth of defective fetuses that otherwise would have died. Excessive amounts of vitamin A can also cause cleft palate (7), and some relevant data from a current experiment (11) with this teratogen will be introduced to aid in the interpretation of the cortisone effect.

Materials and Methods

Mice of the inbred A/Jax strain were mated and females were segregated for treatment if they developed a vaginal plug. Treatment was started at 10 days post conception for vitamin A and at 11 days for cortisone. Vitamin A in oil was delivered through plastic tubing into the stomach in two daily doses of 10,000 units (1/10 cc.) each. Cortisone was injected intramuscularly in doses of 2.5 mg. (1/10 cc.) per day for 4 days. The embryos were recovered at 14 to 16 days or at 18 days post conception. The purposes of the latter collection time was to obtain embryos that would be comparable to newborns without risking the loss of deformed specimens through cannibalism which so frequently occurs if they are allowed to be born. The embryos were fixed *in utero* with Bouin's or Allen's B15 fluid and studied with a dissecting microscope.

Results

The frequencies of cleft lip-cleft palate embryos in litters from treated and untreated female A/Jax mice are given in Table 1. Both cortisone and vitamin A also induce a form of cleft palate not associated with cleft lip, but this aspect of their teratogenic activity will not be described here, since it has already been presented elsewhere in the case of cortisone (4, 6, 12), and will be analyzed in detail later in the case of hypervitaminosis A (7, 11).

Spontaneous cleft lip-cleft palate (CLCP) with a frequency of about 10 to 15% is characteristic of the A/Jax strain. Kalter and Warkany (9) found 12.16% CLCP in their control animals, and since this agrees well with the controls used in this experiment, 12% CLCP has been used as the theoretical expectation in calculating "goodness to fit" for the data in Table 1. A significant reduction in the frequency of CLCP was produced by cortisone treatment but not by vitamin A treatment (Table 1). These differences were observable both at 14–16 days and at 18 days. Litter size was decreased slightly by both teratogens as shown in Table 2. Bilateral cleft lip was more common than either right cleft lip or left cleft lip in untreated litters and in vitamin A treated litters; but not in cortisone treated litters (Table 3).

TABLE 1

Frequency of normal and CLCP embryos in litters from treated and untreated female A/Jax mice

Treatment	Age of embryo (days post conception)	No. with normal lip	No. with CLCP	Percent CLCP	P*
Cortisone	14–16	63	3	4.5	<.001
Cortisone	18	109	5	4.4	<.001
Cortisone	14–16 + 18	172	8	4.4	<.001
Vitamin A	14–16	92	12	11.5	>.3
Vitamin A	18	43	7	14.0	>.3
Vitamin A	14–16 + 18	135	19	12.3	>.3
Untreated	14–16	94	15	13.8	>.3
Untreated	18	89	13	12.7	>.3
Untreated	14–16 + 18	183	28	13.3	>.3

* Probability of deviation from a theoretical 12% CLCP being due to chance.

TABLE 2

Litter size in treated and untreated female A/Jax mice

Treatment	Age of embryos (days post conception)	No. of litters	No. of embryos	Average litter size
Cortisone	14–16	13	66	5.1
Cortisone	18	23	114	5.0
Cortisone	14–16 + 18	36	180	5.0
Vitamin A	14–16	18	104	5.8
Vitamin A	18	10	50	5.0
Vitamin A	14–16 + 18	28	154	5.5
Untreated	14–16	15	109	7.3
Untreated	18	18	102	5.7
Untreated	14–16 + 18	33	211	6.4

TABLE 3

Types of cleft lip found in treated and untreated A/Jax embryos (14 to 18 days old).

Treatment	Number of embryos with		
	Right cleft lip	Left cleft lip	Bilateral cleft lip
Cortisone	5	0	2
Vitamin A	3	6	10
Untreated	6	7	15

Palatine shelf movement, which is normally retarded in CLCP embryos, seems to have been retarded even further by the cleft-palate-producing teratogens (Table 4). At least one shelf reached a horizontal position above the tongue in over half of the untreated CLCP embryos, but in all treated CLCP embryos the tongue was wedged between the shelves, with the shelves being essentially vertical. In two untreated embryos with left cleft lip and with one palatine shelf in the horizontal position dorsal to the tongue, it was the left shelf that had moved to the horizontal position in both cases; the right shelf had remained vertical. Thus, inhibition of palatine shelf movement does not necessarily occur on the same side as the inhibition of lip fusion. One embryo was found with a unilateral cleft lip and a normal palate. This animal was not included in Tables 1 to 4.

TABLE 4

Position of palatine shelves in 18 day old embryos with cleft lip-cleft palate

Treatment	Number of embryos with		
	Both shelves vertical	One shelf vertical, one shelf horizontal	Both shelves horizontal
Cortisone	5	0	0
Vitamin A	7	0	0
Untreated	5	6	2

Discussion

The major effect of cortisone on embryos of the A/Jax strain is the induction of cleft palates (4, 6, 12). In previous publications on the teratogenic effects of cortisone, animals with CLCP (cleft lip-cleft palate) were excluded from the data presented, and the incidence of cleft palate in embryos of A/Jax females treated on the 11th to 14th days post conception was given as 100% (6). In the present study, the percentage of cleft palate, excluding CLCP embryos, is also 100%. If the CLCP embryos are included in the calculations, the embryos with cleft palate due to cortisone constitute 96% and the remaining 4% consist of the "spontaneous" CLCP group. Since the usual frequency of spontaneous CLCP embryos in the A/Jax strain is about 12%, the cortisone is obviously doing more than inducing cleft palate.

It must also be selectively destroying embryos with CLCP, or else "healing" the cleft lips. To comment briefly on the latter possibility, it can be pointed out that the cortisone is given later than the time of normal lip fusion, and the impression gained from studies on palate development (12, 13) is that a normal embryological event probably cannot take place if the usual time for this event has already passed by. A "healing" effect of cortisone also seems unlikely in view of its property of increasing the embryonic mortality rate (Table 2). Thus, it appears reasonable to interpret the decreased frequency of CLCP embryos in litters from cortisone treated females as being due to a differential lethal effect of cortisone on these defective embryos.

If this interpretation is accepted, it raises the problem of why the cortisone would affect CLCP embryos more severely than the other embryos in the litter. It could be reasoned that the CLCP embryos are weaker, and therefore are the first to die under adverse circumstances. However, when Kalter and Warkany (9) gave A/Jax females a riboflavin deficient, galactoflavin (riboflavin antagonist) containing diet, it caused a doubling of the normal resorption rate, but produced no significant change in the frequency of CLCP. Similarly, hypervitaminosis A does not change the frequency of CLCP (Table 1) although it does reduce the litter size about as much as does cortisone (Table 2). It is possible that these two teratogens could destroy the original spontaneous CLCP embryos and replace them with CLCP embryos induced by the teratogens themselves, but the corresponding frequency of 12–15% for both teratogens and normals is too great a coincidence to make this explanation very plausible. Thus, it appears that the CLCP embryos are not more susceptible than normal embryos to a generally adverse environment.

If it is accepted that this lethal action is specific to cortisone then the next factor to consider is cortisone's action on palate closure. Cortisone causes cleft palate by adversely affecting the metabolic processes leading to palatine shelf movement (12). In untreated CLCP embryos, palate movement is also inhibited (Table 4). This is not due to mechanical factors resulting from the presence of a cleft lip, because in the two cases of left cleft lip found in untreated embryos, the shelf on the left side did move to a horizontal position, whereas the shelf on the side with the

normal lip did not move. Actually, this result is logical in view of the mechanics of palate closure (13), because the shelves have to squeeze over the tongue and this would be made easier by the widening of the mouth cavity that results from a cleft lip. Thus, the retardation of shelf movement occurring in untreated CLCP embryos must be due to some defect in the metabolic processes that produce palatine shelf movement. Since cortisone also inhibits these metabolic processes, perhaps it is this combined effect on the same phase of metabolism that proves fatal to the CLCP embryos. The idea of a combined effect on the palate is consistent with the finding that palatine shelves were vertical in all cortisone treated CLCP embryos, but were vertical in less than half of the untreated CLCP embryos (Table 4). The lack of bilateral cleft lip specimens in cortisone treated litters (Table 3) also is consistent with the concept of a weakened metabolic system, since an embryo with both lips cleft must be more deficient in the factors promoting lip fusion than an embryo with only one cleft lip. Yet, the results with hypervitaminosis A seem to challenge the significance of some of these correlations since this treatment also produces cleft palate (and retards shelf movement in CLCP embryos—Table 4) but is not specifically lethal to CLCP embryos (Table 1). However, there are many differences between the actions of cortisone and hypervitaminosis A on the palate (11), so the possibility that cortisone interferes with an already weakened metabolic pathway deserves further study.

Basic to all these considerations is the problem of why only some embryos in a litter develop CLCP when all the embryos supposedly have identical genotypes and very similar environments. The matter of genetic uniformity in an inbred strain is not entirely settled yet. Hayman and Mather (5) claim that a selective disadvantage of the homozygotes could maintain heterozygosity against the pressure of inbreeding. If the genetic basis for CLCP in the A/Jax strain is not gene segregation or mutation, then it presumably involves a strain specific weakness in the factors producing lip closure which would put all embryos close to a threshold, so that adverse fluctuations of the environment could drive a few embryos below this threshold. For cortisone to later act selectively on these same embryos, then either the adverse environment would have to continue to operate on

these few embryos after the cleft lip had developed, or else the metabolic deficiencies which led to the failure of lip fusion would have to persist. Trasler (10) reported that A/Jax embryos in the uterine position nearest the ovary developed spontaneous cleft lip significantly more often than those in other positions in the uterus. Thus, persistently adverse environments within the uterus probably do occur. Regarding the second possibility of a persisting metabolic deficiency, too little is known about the biochemical systems of an embryo to predict whether such systems, if once crippled by a transient adverse environment, would remain crippled for a long period during embryonic development.

Obviously, there is not sufficient information available yet to choose between the various alternative explanations for the specific lethal effect of cortisone. Regardless of the mechanisms involved, the interpretation remains that cortisone destroys certain defective embryos and the withdrawal of cortisone allows survival of these defective embryos. Of course, the withdrawal of cortisone will also reduce the frequency of cleft palates not associated with cleft lip from about 96% to almost 0, so the concurrent rise in CLCP from 4% to 12% is not significant to the overall welfare of the litter. However, the main point of interest in this experiment is not the relative magnitude of the two groups of anomalies, but rather the fact that such a phenomenon can occur experimentally, and therefore might be occurring naturally in human populations. The likelihood of being able to identfy such a factor in the etiology of human congenital anomalies is very slight at present. Fraser (2) has recently discussed the many problems involved in identifying the causative factors in congenital anomalies of human infants. He pointed out that the known environmental and genetic factors account for only a very small percentage of cases, the rest having resisted analysis so far. Also, attempts to translate results from animal experiments to human populations have not, as yet, been profitable. Probably the main contribution of animal experiments has been in providing ideas about what to look for when studying the causes of congenital anomalies in humans.

Summary

Treating pregnant female mice of the A/Jax strain with cortisone caused a decrease in the percentage of embryos with the

"spontaneous" cleft lip-cleft palate anomaly. The teratogenic properties of cortisone were compared with those of hypervitaminosis A, since the two teratogens had similar effects on non-cleft lip A/Jax embryos, but hypervitaminosis A did not reduce the frequency of cleft lip-cleft palate embryos. The lethal effect of cortisone is considered to be of theoretical interest because it can be interpreted as a case in which the withdrawal of a noxious environmental influence caused an increase in the incidence of a particular congenital anomaly.

REFERENCES AND NOTES

1. Davis, M. E., and Edith L. Potter: Congenital malformations and obstetrics. *Pediatrics*, 19: 719, 1957.
2. Fraser, F. C.: Causes of congenital malformations in human beings. *J. Chronic Dis.* 10: 97, 1959.
3. Fraser, F. C., and T. D. Fainstat: Causes of congenital defects. *Am. J. Dis. Child.*, 82: 1, 1951.
4. ————: Production of congenital defects in the offspring of pregnant mice treated with cortisone. *Pediatrics*, 8: 527, 1951.
5. Hayman, B. I., and K. Mather: The progress of inbreeding when homozygotes are at a disadvantage. *Heredity*, 7: 165, 1953.
6. Kalter, Harold: The inheritance of susceptibility to the teratogenic action of cortisone in mice. *Genetics*, 39: 185, 1954.
7. Kalter, Harold, and Josef Warkany: Teratogenic action of hypervitaminosis A in strains of inbred mice. *Anat. Rec.*, 133: 396, 1959.
8. ————, and ————: Experimental production of congenital malformations in mammals by metabolic procedure. *Physiological Reviews*, 39: 69, 1959.
9. ————, and ————: Congenital malformations in inbred strains of mice induced by riboflavin-deficient, glaactoflavin-containing diets. *J. Exp. Zool.*, 136: 531, 1957.
10. Trasler, Daphne G.: The influence of uterine position and other factors on the occurrence of spontaneous cleft lip in mice. *Records Genetics Soc. Amer.*, 28: 102, 1959.
11. Walker, B. E., and Burton Crain, Jr.: Unpublished observations.
12. Walker, B. E., and F. C. Fraser: The embryology of cortisone-induced cleft palate. *J. Embryol. exp. Morph.*, 5: 201, 1957.
13. ————, and ————: Closure of the secondary palate in three strains of mice. *J. Embryol. exp. Morph.*, 4: 176, 1956.
14. Warkany, Josef: Congenital malformations and pediatrics. *Pediatrics*, 19: 725, 1957.

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BOOK REVIEWS

For the record, three *Antibiotics Monographs* must be noted: Hirsh, Harold L. and Lawrence E. Putnam: *Penicillin.* Antibiotics Monographs no. 9. Medical Encyclopedia, Inc., New York, N. Y. 1958.—Knowledge in 1958 in regard to development and commercial production of penicillin, its antimicrobial activity, pharmacology, dosage forms, and its use in pneumococcal infections, staphylococcal infections, meningococcal and gonococcal infections, miscellaneous infections and viral infections is summarized, with a brief discussion of penicillin V. The bibliography comprses 159 papers.

Weinstein, Louis and N. Joel Ehrenkranz: *Streptomycin and Dihydrostreptomycin.* Antibiotics Monograph no. 10. Medical Encyclopedia, Inc., New York, N. Y. 1958.—Knowledge in 1958 is summarized as to history and development, chemical and biological properties, sensitivity-testing and assay methods, resistance, dependence, synergism and hormesis, pharmacology, untoward effects, use in bacteremia and endocarditis, meningitis due to Gram-negative bacilli, in respiratory infections, brucellosis, shigellosis, tularemia, g.-u. infections, plague, surgical infections, ophthalmology, ear infections and miscellaneous infections, and brief notes on diseases unaffected by these agents, combinations with other antibiotics, and use of these agents in isolation of viruses. 460 papers are included in the bibliography.

Mitchell, Roger S. and J. Carroll Bell: *Modern Chemotherapy of Tuberculosis,* Antibiotics Monographs no. 11. Medical Encyclopedia, Inc., New York, N. Y. 1958.—Knowledge in 1958 of history; pharmacology of streptomycin (SM), *p*-aminosalicylic acid (PAS), isoniazid (INH) and accessory or secondary drugs; modes of action of SM, PAS, INH and combinations; dosages of SM, PAS, INH and other drugs; 8 regimens studied; drug resistance; therapeutic recommendations ("Modern Chemotherapy of Tuberculosis"); diseases and conditions having an adverse effect upon the course of chemotherapy of tuberculosis; chemotherapy with new and less-used drugs; and results of antituberculosis chemotherapy is summarized. 510 papers are noted in the bibliography.

G. A. Emerson

Ciba Foundation Symposia, edited by G. E. Wolstenholme and Cecelia M. O'Connor. Little Brown, Boston, Mass. and J. and A. Churchill Ltd., London. Regulation of Cell Metabolism. 387 pp., $9.50. Neurological Basis of Behavior. 400 pp., $9.00.

The regulation of cell metabolism is discussed by 39 active workers largely from England, Germany and the United States and includes four Nobelists. The sixteen papers presented are biochemical with the exception of one electron microscope analysis of cell components. Attention is given to localization and the grouping of enzymes in microsomes, lyzosomes and mitochondria as well as limiting membranes bordering the cell or its parts. Active transport

is seriously considered. Comparison between the self regulating cellular mechanisms and electrical feedback is debated. The symposium is a well rounded picture of the present status of cell metabolism and points specifically to unknowns that need investigation.

The neurological basis of behavior is examined by 34 leading investigators in honor of the centennial of the birth of Sir Charles Sherrington. The United States and England contribute one third each and the rest come from France, Switzerland, Sweden, South Africa and Australia. A preliminary chapter on mind-brain theories is followed by J. C. Eccles on behavior of nerve cells. Most of the discussion centers about the reticular core of the upper brain stem, its relation to thalamic and hypothalamic centers and the general role of the archipallium. The net conclusion of the symposium is that while rapid progress is being made in different attacks the central process of thinking and learning as related to behavior is still to be found. It is very probable that in animal subjects as in man there may be a lot of cerebration without measurable response so that response may not be used as a measure of learning or thinking. Dr. Penfield points to the great value of studying brains in epilepsy under natural experimental conditions that could not be duplicated. Brain waves are still statistical summations difficult to interpret. Biochemical approaches are mostly for the future.

John G. Sinclair

Principles of Pathology, by Howard C. Hopps, M.D., The University of Texas Medical Branch, Galveston, Texas. Appleton-Century-Crofts, New York, N. Y., 1959. 301 pages, 613 figures. $6.95.

This book is a monument to the art of teaching basic principles with a combination of simplified illustrations and well chosen words. The illustrations consist of black and white stippled line drawings which are arranged vertically along the outer margin of each page. Many of the drawings are unlabelled and serve to catch the eye, arouse the interest and add life to the adjacent paragraph. A blood count, a quotation, a chemical formula, and a temperature chart here and there give the illustrations a clinical touch. The style of writing is conversational with some pointed and sophisticated statements to prevent the student from thinking that he will master the subject of pathology in the time it takes to read this book. The sentences are not interrupted by distracting notations or references. At the end of each chapter a few references, notably monographs and books, are listed for further reading.

The book begins with an historical sketch and proceeds immediately into a broad treatment of circulatory disturbance. Thus, the book begins with hemorrhage—a dramatic subject which serves as a beginning for the discussion of reaction to injury. The general principles are illustrated by specific examples. There are only six chapters in the book as follows: (1) The pathologic approach to disease (2) circulatory disturbances (3) disturbances of growth and development (excluding neoplasia) (4) degenerative processes and disorders directly related to metabolism (5) inflammation (6) neoplasia.

Each chapter is approximately 50 pages long and is subdivided into well balanced sections. An appendix (5 pages) includes a table of normal weights and measures of organs, a table of normal values of clinical laboratory tests, prefixes commonly used in pathology, and some basic considerations of fixation, dehydration and other techniques in the preparation of histologic sections.

This book is written for the medical student, but physicians, including specialists in pathology, will find it to be an excellent review. The book makes no attempt to cover "system pathology" and most medical school courses in pathology will find that a second book with more details will be necessary for additional reading and for advanced pathology. This book could be used effectively for courses to graduate students, nurses and students in paramedical fields.

The book is well printed and well bound.

K. M. Earle

William C. Gibson: *Young Endeavour*. Springfield, Ill. Charles C Thomas, 292 pp.

This Liebeswerk of the Kinsmen Professor of Neurological Research at the University of British Columbia carries the subtitle "Contributions to Science by Medical Students of the Past Four Centuries." For the present mobilization of scientific effort, implicating more medical students in research, this book is very timely. The personal touches in the lives of Blake, Ramon y Cajal, Cushing, the various Hopkins, University College- and Harvard- men among others, are enhanced by the author's wide roots from Madrid to Oxford, and his intimacy with such men as Chauncey Leake, John Fulton, and Sir Henry Dale, who wrote the foreword.

Living men are excluded, perhaps unnecessarily. Omissions, e.g., Korotkov, are inevitable, as are overstressed biographies. Style and format are adequate, if choppy. Quotations, photographs and translations abound in the text, which is followed by general references and an index.

The forcible impression is left that medical scientists, like physicists and mathematicians, are apt to show their genius prior to age 25. Their example, the author correctly hopes, will "help us, students and teachers alike, to be more imaginative investigators of nature."

Arthur Ruskin

The Mast Cell. James F. Riley. E. & S. Livingstone, Ltd., Edinburgh, 1959. 182 pp. Illus. $4.25.

It has become increasingly apparent that tissue mast cells may play an important role in the physiopathology of connective tissue. This thesis is discussed in Dr. Riley's book which contains an excellent review on mast cells in tumors, chronic and acute inflammations, anaphylaxis, peptone shock, etc., which is used to introduce the author's own work on the presence of histamine in the mast cells and its release by histamine liberators. The release of heparin from the mast cell and its fate and function in the tissues

are also discussed. Unfortunately no mention is made of the ability of heparin to detoxify several of the histamine liberators, including compound 48/80. The toxicity of this latter substance in the rat, according to Dr. Riley, is mediated solely through its interaction with mast cells and the consequent release of histamine. A "mast cell cycle" has been proposed by the author and it is of interest to find that it contains many elements of this reviewer's concept of a detoxifying process in connective tissue called "micellophagosis."

R. D. Higginbotham

CALLING ATTENTION TO

For Nov Centennial of Darwin's Origin of Species: N Barlow edits *The Autobiography of Charles Darwin, 1809–1882* (Harcourt, Brace, NY, 59, 263 pp, $4.5). HG Cannon clarifies *The Evolution of Living Things* (CC Thomas, Springfield, Ill, 59, 190pp, $3.5). B Glass edits *Forerunners of Darwin* (Johns Hopkins Univ Press, Baltimore, 59, 479pp, $6.5). M Peckham gives variorum text of *The Origin of Species by Charles Darwin* (Univ of Pennsylvania Press, Philadelphia, 59, 670pp, $15). A Roe & GG Simpson edit *Behavior & Evolution* (Yale Univ Press, New Haven, Conn, 59, 466pp, $10). C Zirkle reviews *Evolution, Marxian Biology & the Social Scene* (Univ Pennsylvania Press, Philadelphia, 59, 520pp, $7.5). Librarians note: Get these books for exhibit in autumn.

Clinical: F Boys & HM Smith discuss *Poisonous Amphibians & Reptiles: Recognition & Bite Treatment* (CC Thomas, Springfield, Ill, 59, 164pp, $5). JJ Byrne goes over *The Hand: Its Anatomy & Diseases* (ibid, 408pp, $10.5). CC Chapple edits *Research in Aging* (VA Adm, US Govt Printing Office, Washington DC, 59, 125 pp, $1.5). CH Delacato describes *The Treatment & Prevention of Reading Problems* (CC Thomas, Springfield, Ill, 59, 136pp $4.5). SD Elek is timely in *Staphlococcus Pyogenes & Its Relation to Disease* (Williams & Wilkins, Baltimore, 59, 773pp, $15). CW Lloyd edits *Endocrinology of Reproduction* (Academic Press, NY, 59, 532pp, $12). HS Lawrence edits *Cellular & Humoral Aspects of the Hypertensive States* (Hoeber, NY, 59, 679pp, $18). AE Mourant analyses *The ABO Blood Groups* (CC Thomas, Springfield, Ill, 59, 284pp, $8.5). JH Moyer edits *Hahnemann Symposium on Hypertensive Disease* (WB Saunders, Philadelphia, 59, 800 pp, $14). V Najjar edits *Immunity & Virus Infection* (Wiley, NY, 59, 270pp, $10.5). IH Page edits *Connective Tissue, Thrombosis & Atherosclerosis* (Academic Press, NY3, 59, 460pp, $11). HE Stephenson Jr informs on *Cardiac Arrest & Resuscitation* (CV Mosby, St. Louis, Mo, 59, 378 pp, $12). HA Teloh tells about *Clinical Flame Photometry* (CC Thomas, Springfield, Ill, 59, 120pp, $4.5). PG Weil describes *The Plasma Proteins: Clinical Significance* (Lippincott, Philadelphia, 59, 260pp, $6).

From Industry: F Bello well describes E (Polaroid) Land's disturbing findings on color vision & perception (*Fortune*, May 1959, pp 144–148). E Von Fange, from staff experience at General Electric, analyses *Professional Creativity* (Prentice-Hall, Englewood Cliffs, NJ, 59, 260pp, $5).

Preclinical Science: D Bovet & Co well consider *Curare & Curare-like Agents* (VanNostrand, Princeton, NJ, 59, 489pp, $16). J Brachet & AE Mirsky edit *The Cell: Biochemistry, Physiology, Morphology*, to appear in 3 vols—Vol 1 on methods & cell biology now ready (Academic Press, NY3, 59, 780pp, $22). TH Bullock reviews neuron doctrine & electrophysiology (*Science* 129/997/Apr 17/59). FM Burnet & WM Stanley edit *The Viruses: Biochemical, Biological & Biophysical Properties* (Academic Press, NY3, 59, 3 vols, 670, 530, 415 pp, about $45). L Chertok & P Kramarz in reviewing hypnosis & sleep in relation to EEG show wide variation in objective criteria of hypnosis (*J Nerv*

Ment Dis 128/227/59). M Gordon edits *Pigment Cell Biology* (Academic Press, NY3, 59, 647pp, $13.5). N Kamiya describes *Protoplasmic Streaming* (Vol 8 Protoplasmatologia, Lange & Springer, Berlin-Wilmersdorf, 59, 210 pp, DM54). FNL Poynter edits *The History & Philosophy of Knowledge of the Brain & Its Functions* (CC Thomas, Springfield, Ill, 59, 284 pp, $5.5). JM Reiner discusses *Behavior of Enzyme Systems: An Analysis of Kinetics & Mechanism* (Burgess Publ, Minneapolis 15, Minn, 59, 325pp, $6.5). F Sanger reviews chemistry of insulin (*Science* 129/1340/May 15/59). HK Schachman describes *Ultracentrifugation in Biochemistry* (Academic Press, NY3, 59, 280pp, $8.8). WA Waters edits memorial vol to MS Kharasch on *Vistas in Free Radical Chemistry*, of growing importance in biology (Pergamon Press, NY22, 59, 200pp, $12.5).

General: DA Barbara rings a bell with *The Art of Listening* (CC Thomas, Springfield, Ill, 59, 208pp, $5.5). J Barzun builds *The House of Intellect* (Harper, NY, 59, 312pp, $5). D Bindre tackles *Motivation: A Systematic Reinterpretation* (Ronald Press, NY, 59, 368pp, $5.5). C Brinton gets into *A History of Western Morals* (Harcourt, Brace, NY, 59, 512pp, $7.5). GW Elbers & P Duncan edit *The Scientific Revolution: Challenge & Promise* (Public Affairs Press, Washington, DC, 59, 288p, $6). MC Hope edits important *Proceedings of the National Conference on Air Pollution* (US Public Health Service Publ. No 654, US Govt Printing Office, Washington, DC, 59, 526pp, $1.75). A Koestler is provocative in *The Sleep Walkers: A History of Man's Changing Vision of the Universe* telling about classic astronomers (Macmillan, NY, 59, 624pp, $6.5). L Lipton digs cool cats in *The Holy Barbarians* (Messner, NY, 59, 318pp, $5). LJ Rather translates with keen introduction selected essays by R Virchow (1821–1902) in *Disease, Life & Man* (Stanford Univ Press, Stanford, California, 59, 273pp, $5).

Books of more than passing interest: AD Bass edits timely *Evolution of Nervous Control from Primitive Organisms to Man* (AAAS, Washington 5, DC, 59, 231pp, $6). JC Belisario well surveys *Cancer of the Skin* (Butterworth, London, 59, 335pp, 50s). HF Blum is startling in *Carcinogenesis by Ultraviolet Light: An Essay in Quantitative Biology* (Princeton Univ Press, Princeton, NJ, 1959, 376pp, $6). M Boas well relates *Robert Boyle & 17th Century Chemistry* (Cambridge Univ Press, NY, 59, 412pp, $7.5). TN Bonner describes *The Kansas Doctor: A Century of Pioneering* (Univ Kansas Press, Lawrence, 59, 260pp, $5). Sir Russell Brain indicates *The Nature of Experience* (Oxford Univ Press, NY, 59, 88pp, $2). R Coulborn considers *The Origin of Civilized Societies* (Princeton Univ Press, Princeton, NJ, 59, 228 pp, $4). I Galdston introduces GS Pondoev's *Notes of a Soviet Doctor* (Consultant's Bureau, NY11, 59, 210pp, $5). BW Halstead describes *Dangerous Marine Animals* (Cornell Maritime Press, Cambridge, Md, 59, 152pp, $4). NR Hanson well outlines *Patterns of Discovery: An Inquiry into the Conceptual Foundations of Science* (Cambridge Univ Press, NY, 58, 251pp, $5.5). Magnificent is WS Heckscher's *Rembrandt's Anatomy of Dr. Nicholas Tulp* (New York Univ Press, NY, 59, 283pp, $15). CL Hubbs edits important *Zoogeography*, a series of AAAS symposia, dedicated to the memory of Darwin & Wallace (AAAS, Washington 5, DC, 59, 520pp, $12). A & M Keys say *Eat Well & Stay Well* (Doubleday, NY, 59, 321pp, $4). ACB Lovell

relates *The Individual & the Universe* (Harper, NY, 59, 111pp, $3). T Mahoney praises *The Merchants of Life: An Account of the American Pharmaceutical Industry* (Harper, NY, 59, 285pp, $3.75). H Mehling enjoys *The Scandalous Scamps* (H. Holt, NY, 59, 211pp, $4). W Penfield & L Roberts go to town on *Speech & Brain Mechanisms* (Princeton Univ Press, Princeton, NJ, 59, 286pp, $6). JA Perkins offers *Plain Talk from a Campus*, commenting keenly on USA higher education (Univ Delaware Press, Newark, Del, 59, 205pp, $4). George Sarton's great *History of Science* goes on, in *Hellenistic Science & Culture in the Last 3 Centuries BC*, with fine account of Alexandrian Library as noted by J Lear in *Saturday Review* of June 20/59 (Harvard Univ Press, Cambridge, Mass, 59, 554pp, $11). May Sarton goes autobiographical in *I Knew a Phoenix* (Rinehart, NY, 59, 268pp, $3.75).

Grammatolator JE Schmidt goes to town: (1) *Dictionary of Medical Slang & Related Esoteric Expressions;* (2) *Medical Discoveries: Who & When*, and (3) *Narcotics: Lingo & Lore*, a snappy dictionary (all published by CC Thomas, Springfield, Ill, 59, respectively 224pp, $7; 540pp, $15 & 216pp, $4.25).

Medical Technology: JJ & MJ Edwards neatly discuss *Medical Museum Technology* (Oxford Univ Press, NY, 59, 182pp, $3.4). TC Ruch well describes *Diseases of Laboratory Primates* (WB Saunders, Philadelphia, 59, 600pp, $12).

Psychology & Psychiatry: M. Balint considers primitive attitudes in *Thrills & Regressions* (International Universities Press, NY, 59, 148pp, $4). E Bergler stresses pleasure in displeasure patterns in *Principles of Self-Damage* (Philosophical Library, NY, 469pp, $6). FJ Braceland edits important reports on *The Effects of Pharmacologic Agents on the Nervous System* (Vol 37, Research publications *Asso. Nerv. Ment. Dis.*, Williams & Wilkins, Baltimore, Md, 59, 488pp, $13.5). H. Gulliksen shows how mathematics helps psychology (*Amer. Scientist*, 47/178/59). S Hook edits *Psychoanalysis, Scientific Method & Philosophy* (New York Univ Press, NY, 59, 260pp, $5). CL Li notes that units of cerebral cortex seldom fire synchonously, tho there is temporal relationship, even in arousal (*Science* 129/783/Mch 20/59). G Mandler & W. Kessen consider *The Language of Psychology* (J Wiley, NY16, 59, 312 pp, $7). Lord Pakenham examines *Causes of Crime* (CC Thomas, Springfield, Ill, 59, 198pp, $4.75). B Passamanick & P. K. Knapp edit *Social Aspects of Psychiatry* (American Psychiatric Asso., Washington, DC, 59, 208pp, $2). WL Warner studies American Symbolism in *The Living and the Dead* (Yale Univ Press, New Haven, Conn, 59, 410pp, $7.5). BB Wassell suggests methodology for *Group Psychoanalysis* (Philosophical Library, NY, 59, 306pp, $3.75). P Weiss looks into *Our Public Life*, analysing our society (Indiana Univ Press, Bloomington, Ind, 59, 244pp, $4.5). RJ Williams pushes on in *Alcoholism: Its Prevention by Nutrition* (Univ Texas Press, Austin, Tex, 59, 108pp, $2.5).

Clinical: JA Anderson edits memorial contributions to Erling S. Platou (1896–1958), distinguished pediatrician (*Jour-Lancet*, 79/219–289/59). JE Birren, HA Imus & WF Windle edit important volume, *The Process of Aging in the Nervous System* (CC Thomas, Springfield, Ill, 59, 240pp, $7). HT Blumenthal & JG Probstein summarize long study of *Pancreatitis* (CC

Thomas, Springfield, Ill, 59, 392pp, $10). A Gelperin advises physician-pharmacist team-work in drug evaluation (*Amer. J. Hosp. Pharm.*, 16/217/59). JW Gofman shoots his wad on *Coronary Heart Disease* (CC Thomas, Springfield, Ill, 59, 374pp, $8). F Gollan discusses *Physiology of Cardiac Surgery* (CC Thomas, Springfield, Ill, 59, 104pp, $4.5). GM Grodsky & CT Peng show tolbutamide does not decrease insulin in pancreas (*Proc Soc Exp Biol Med*, 101/100/59). FW Heggeness finds galactose & lactose depress volume flow of bile (*ibid* p 143). EC Hoff offers a critical bibliographical review of *The Bromides* (Interscience Publications, NY, 59, 212pp, $6). KYT Kao & TH McGavack show increase with age of scleroprotein in skin, tendons, uterus & aorta (*Proc Soc Biol Med*, 101/153/59). SS Kety offers keen critique of biochemical theories of schizophrenia (*Science* 129/1528, 1590/June 5 & 12/59). LM Neuschutz outlines *Vocational Rehabilitation for the Physically Handicapped* (CC Thomas, Springfield, Ill, 59, 154pp, $5.75). H Peron & MH Jones debate nervous pathways of cutaneous pain (*Science* 129/1547/June 5/59). CJ Polson gives *The Essentials of Forensic Medicine* (CC Thomas, Springfield, Ill, 59, 272 pp, $7.5). B Shartel & ML Plant state *The Law of Medical Practice* (CC Thomas, Springfield, Ill, 59, 468pp, $12.5). PK Smith compiles a critical bibliographical review of *Acetophenetidin* (Interscience Publ., NY, 59, 180pp, $5.75). N Thal describes cumulative index of anti-depressants, recommending b-phenyl-ethyl-hydrazine, "Nardil" (*Dis Nerv Syst*, 20/197/59). T Winsor tries objective approach to *Peripheral Vascular Diseases* (CC Thomas, Springfield, Ill, 59, 864pp, $16.5). HA Zimmerman edits *Intravascular Catheterization* (CC Thomas, Springfield, Ill, 59, 768pp, $17).

Of Utmost Importance is condemnation of official attitudes toward drug addicts, made by Judge JM Murtagh & S Harris in *Who Live in Shadow* (McGraw-Hill, NY, 59, 207pp, $4.5).

Some Interesting New Books: M Amrine raises serious moral questions in *The Great Decision: the Secret History of the Atomic Bomb* (Putnam's, NY, 59, 251pp, $4). PW Bridgman notes operational methodology in *The Way Things Are* (Harvard Univ Press, Cambridge, Mass, 59, 343pp, $5.75). RA Brown edits keen essays *On Translation* (*ibid*, 297pp, $6.5). M Bunge raises *Metascientific Queries*, for tender-minded metaphysicians & truculent scientists (CC Thomas, Springfield, Ill, 59, 328pp, $7). R Coulborn describes *The Origin of Civilized Societies* (Princeton Univ Press, Princeton, NJ, 59, 231pp, $4). B Decaux considers *Le Mesure Précise du Temps* (Masson, Paris, 59, 126pp, 1300 Fr). M & A Edel skillfully relate *Anthropology & Ethics* (CC Thomas, Springfield, Ill, 59, 264pp, $5.5). R Eriksson nicely translates & edits Baldasar Heseler eyewitness report on *Andreas Vesalius' First Public Anatomy at Bologna 1540* (Sandberg's Bokhandel, Stockholm, 59, 344pp, $11). G Hummelfarb adds *Darwin & the Darwinian Revolution* (Doubleday, Garden City, NY, 59, 314pp, $6). ML King Jr takes big stride *Toward Freedom* in describing passive resistance to segregation (Harper, NY, 58, 188pp, $3). LR Lind & PG Roofe annotate J Berengario da Carpi's Bologna 1523 *Short Introduction to Anatomy* (Univ Chicago Press, Chicago 37, Ill, 59, 239 pp, $5. H Livermore writes timefully *A History of Spain* (Farrar, Straus & Cudahy, NY, 59, 478pp, $6.5). A Maurois gives his usual fine treatment to

The Life of Sir Alexander Fleming, the Discoverer of Penicillin (Dutton, NY, 59, 292pp, $5). CW Mills critically examines sociology in *The Sociological Imagination* (Oxford Univ Press, NY, 59, 342pp, $6). Smartyping M Natenberg does some uncritical potentially dangerous help-the-underdog reporting of cancer quackery in *The Cancer Blackout* (Regent House, Chicago, Ill, 59, 206pp, $4). RG Richardson tells *The Surgeon's Tale*, a history of modern surgery (Scribner's, NY, 59, 266p, $5). P Toynbee conducts debate on nuclear policy in *The Fearful Choice* (Wayne State Univ Press, Detroit, Mich, 59, 112pp, $2.5). J Thurber has a bang from *The Years with Ross* (Little, Brown, Boston, Mass, 59, 310pp, $5). V. Zuckerkandl studies *The Sense of Music* (Princeton Univ Press, Princeton, NJ, 59, 252pp, $6).

Toxicology: JM Barnes & HB Stoner survey toxicity of tin compounds (*Pharmacol Rev* 11/211/59). K Lohs discusses *Synthetische Gifte* (Deutscher Buch Export, Leipzig C 1, 59, 216pp, 10 DM).

Clinical: NWH Addink & JLP Frank find subnormal zinc blood levels in cancerous patients (*Cancer* 12/544/59). G Barlow & RL Wooton note reduced plasma Na, K, & protein in chronic alcoholics (*Proc Soc Exp Biol Med* 101/44/59). HF Blumm in his address to pharmacologists on quantitative aspects of cancer induction & growth, indicates inevitability of carcinogenesis if living material lives long enough (*Amer Sci* 47/250/59). GL Engel reviews psychogenic pain & pain-prone patients (*Amer J Med* 26/899/59). GWN Eggers Jr & Co note phenothiazines do not alter vasopressor responses to norepinephrine (*Anesth* 20/261/59). L Gyorgy & Co describe "Chinoparine" (dimethyl-6,7-dimethoxy-isoquinoline) as spasmolytic more powerful & less toxic than papaverine (*Acta Physiol Acad Sci Hung* 15/189/59). S Hajdu & E Leonard analyse cellular basis of cardiac glycoside action (*Pharmacol Rev* 11/173/59). WH Havener & Co are skeptical about heparin therapy in senile macular degeneration (*Arch Ophthal* 61/390/59). HE Himwich & Co report imipramine HCl (Tofranil, Geigy) has chlorpromazine-like blocking of pain arousal & EEG sleep pattern, but is stimulating against psychotic depression (*Proc Amer Psychiat Asso*, Phila, May, 59). K Konno & Co conclude x-irradiation & aging have similar effects on collagen metabolism (*Proc Soc Exp Biol Med* 101/320/59). RS Ledley & LB Lusted show how symbolic logic, probability & value theory aid in understanding how physicians reason in diagnosing (*Science* 130/9/July 3/59). JT Martin & Co review EEG in anesthesia (*Anesth* 20/359/59). W Penfield shows how stream of consciousness in human brains can be electrically reactivated (*Science* 129/1719/June 26/59). JM Price & Co show abnormal tryptophan metabolism in porphyria & schizophrenia (*Neurol* 9/456/59). DW Talmadge reviews immunological specificity (*Science* 129/1643/June 19/59). SO Waife & AP Shapiro edit *Clinical Evaluation of New Drugs* (Hoeber, NY, 59, 223pp, $7.5). W Weislo analyses hemodynamics of histamine shock showing cardiac failure (*Acta Physiol Polonica* 10/141/59).

PRECLINICAL: R Benesch & Co edit symposium on *Sulfur in Proteins* (Academic Press, NY, 59, 469pp, $12). FB Benjamin believes pain summation to be characteristic of pain sensation (*Proc Soc Exp Biol Med* 101/380/59). E Bulbring & A Crema find intestinal pressure increase releases 5-hydrosytryptamine & that 5HT facilitates peristalic reflexes (*J Physiol* 146/18,29/59). C

Coers & AL Woolf describe biopsy studies of *The Innervation of Muscle* (CC Thomas, Springfield, Ill, 59, 166pp, $9). CE Cornelius & Co show inherited muscular dystrophy in chickens goes with increased aldase & glutamic oxaloacetic-transaminase activity (*Proc Soc Exp Biol Med* 101/41/59). DR Curtis & Co find gamma-amino-butyric acid & beta alamine have non-specific depressant action on surface membranes of neurones (*J Physiol* 146/185/59). HM Gershenfeld & Co find no extracellular space or intercellular ground substance in central nervous system & that astroglia function as water-ion compartments for selective transport between blood & neurones (*Neurol* 9/412/59). GV Gershuni summarizes the Oct 1958 Moscow colloquium on electroencephalography of higher nervous activity (*Sechenov Physiol J USSR* 45/189/59). M Gordon edits *Pigment Cell Biology* (Academic Press, NY, 59, 647pp, $13.5). EM Grook edits *The Structure & Function of Subcellular Components* (Cambridge Univ Press, NY, 59, 101pp, $4.25). T Hayashi edits *Subcellular Particles* (Ronald Press, NY, 59, 213pp, $6). BN Halpern reviews the role & function of reticulo-endothelial system in immunological processes (*J Pharm Pharmacol* 11/321/59). H Jackson shows wide range of antifertility agents (*Pharmacol Rev* 11/135/59). NA Kelareva indicates that cardiac depolarization & repolarization are independent of glycolysis (*Sechenov Physiol J USSR* 45/67/59). O Krayer & G Koelle edit important NIH symposium on *Catecholamines*, arranged by BB Brodie & Co (*Pharmacol Rev* 11/241–566/59, & separate). J Lederberg surveys genes & antibodies (*Science* 129/1649/*June* 19/59). H Peeters edits *Protids of the Biological Fluids* (Van Nostrand, Princeton, NJ, 59, 339pp, $8.5). DN Stern & EM Stuni note tissue taurine is not source of excess taurine in urine after irradiation (*Proc Soc Exp Biol Med* 101/120/59). GK Stookey & JC Muhler find molybdenum increases availability of fluoride ion in preventing dental caries (*ibid* p379). T Szilagyi & Co note that hypothermia blocks histamine release (*Acta Physiol Acad Sci Hungar* 15/69/59). WW Tourtellotte finds higher sphingomyelin & phospholipids in cerebrospinal fluid of men than of women, but lower cholesterol (*Neurol* 9/375/59).

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